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VOLUME 95 NUMBER 1 September 1975

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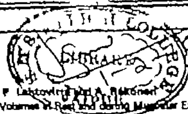
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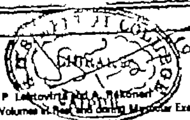
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liter milliliter micro-liter	l ml µl
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Editorial office

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...Astra Läkemedel AB, Södertälje, Sweden
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Acta physiol. scand. 1975. 95. 1 5

From the Research and Development Laboratories, Astra Läkemedel AB, Södertälje, Sweden and Research and Development Laboratories, AB Draco, Lund, Sweden

The Biliary Excretion of ³H Inulin and ³H Terbutaline in the Unanesthetized Rat

By

HANS ERIKSSON, WERA HELLSTRÖM and ÅKE RYRFELDT

Received 10 December 1974

Abstract

ERIKSSON, H., W. HELLSTRÖM and Å. RYRFELDT. *The biliary excretion of ³H-inulin and ³H terbutaline in the unanesthetized rat.* Acta physiol. scand. 1975. 95. 1 5

An animal model which permits sampling of arterial blood and bile from unanesthetized rats with uninterrupted enterohepatic circulation is described. Pharmacokinetic data concerning inulin (0.2 μmol kg⁻¹) and terbutaline (1.8 μmol kg⁻¹) after intraarterial injection are presented. The following values for inulin were obtained Vdβ 0.25 ml g⁻¹ plasma clearance = 2.3 ml min⁻¹ kg⁻¹ and bile clearance = 0.03–0.04 ml min⁻¹ kg⁻¹ which mainly are in agreement with data from the literature. For terbutaline the corresponding values were 0.58 ml g⁻¹ 5.2 ml min⁻¹ kg⁻¹ and 0.12–0.98 ml min⁻¹ kg⁻¹ respectively.

In most studies concerning biliary excretion in rats anesthetized bile fistula animals have been used. The anesthesia and trauma of the operation may effect the excretory function (Herman *et al.* 1971). The use of unanesthetized rats, which have been allowed to recover from the operation might therefore be of advantage. Various techniques to obtain unanesthetized bile fistula rats with or without interruption of the enterohepatic cycle have been described (Lambert 1965). From a physiological point of view an animal model without interrupted enterohepatic circulation is to be preferred (Light *et al.* 1959). The knowledge concerning biliary clearance and excretion using unanesthetized rats is scanty. In the present investigation the biliary excretion of inulin and terbutaline have been studied using the unanesthetized bile fistula rat, with intact enterohepatic circulation.

Inulin is a polar high molecular compound known to have a low biliary clearance (Schanker and Hogben 1961). Terbutaline, an antiasthmatic agent of the β_2-stimulating type, is a highly polar amphoteric compound. The cumulative biliary excretion of this drug has been reported recently (Nilsson *et al.* 1973).

Materials and Methods

Compounds

Tritiated ³H-terbutaline sulphate [1-(3,5-dihydroxyphenyl)-2-(t-butylamino)-1-³H-ethanol sulphate] was synthetized at the Research Laboratories, Astra Läkemedel AB, Södertälje, Sweden. Tritiated inulin, randomly labelled, was obtained from the Radiochemical Centre, Amersham, England. The molar radioactivity of terbutaline and inulin was 13.6 and 0.3 Ci mmol⁻¹ respectively. The radiochemical purity of ³H-terbutaline was found to be higher than 0.95 according to thin layer chromatography analysis (see below). The radiochemical purity of inulin was about 0.98 according to the manufacturer.

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Figures should not be larger than manuscript pages and sent in as glossy prints in a size lar that required for reproduction. Lettering should be large enough to permit suitable redu preferably of uniform size. When possible diagrams and photomicrographs should extend h ly rather than vertically in order to save space Photomicrographs should be calibrated w *(not as enlargement factor in figure text) Figure texts should be assembled on separate she*

Tables should be kept at minimum both in number and size with text above the tabl separate sheets). Single numbers in a series should be replaced by mean and S.D. or mean in the latter case with number of observations

Key words (5–10) are recommended in order to facilitate indexing.

For abbreviations units and symbols see special list in the Journal and recent articles.

More detailed instructions to authors are under preparation pending the recommendation Scandinavian Publications Committee.

The international system of units (SI)

The following symbols and units recommended by the SI are being used in Acta Ph Scandinavica Certain units not included in SI will still be permitted

SI units with recommended symbols

Units	*Symbols*
kilogramme	kg
second millisecond	s ms
mole millimole micro-mole nanomole picomole	mol mmol µmol nmol pmol
meter millimeter micrometer nanometer	m mm µm nm
candela	cd
steradian	sr
hertz (frequency)	Hz (s^{-1})
newton (force)	N (kg m/s^2)
pascal (pressure)	Pa (N/m^2)
joule (energy)	J (N m)
watt (effect)	W (J/s)
lumen (lightflow)	lm (cd sr)
lux (illumination)	lx (lm/m^2)

Conversion factors to be given in Methods.

Permitted non-SI units

Units	*Symbols*
gramme	g
minute	min
hour	h
molarity (mol/liter)	M
(calorie)	cal (4 184 J
(kilopond)	kp (9.81 N)
(millimeters of mercury)	mm Hg (1.333 bar)
(millibar)	mbar (100
curie	Ci
liter milliliter microliter	l ml µl
degree Celsius	°C

ABLE II. Some pharmacokinetic parameters for inulin (0.2 μmol kg^{-1}) and terbutaline (1.8 μmol kg^{-1}) administered intraarterially to unanesthetized male bile fistula rats. A two compartment open model has been used to characterize the pharmacokinetic profiles of the compounds.

arameter	Inulin	Terbutaline
	8.62	34.5
(nmol ml^{-1})	0.18	0.27
(nmol ml^{-1})	7.22	6.42
(h^{-1})	0.54	0.56
(h^{-1})	0.25	0.38
ℓβ (ml g^{-1})	2.3	5.2
lasma clearance (ml min^{-1} kg^{-1})	0.03–0.04	0.13–0.98
ile clearance (unchanged) (ml min^{-1} kg^{-1})	0.1	1.2
rythrocyte/plasma ratio		

1971) anesthesia and trauma of operation may affect the excretory function. Further xperiments are therefore needed in order to clarify differences in excretory function etween anesthetized and unanesthetized animals.

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References

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DICKER, S. E. and H. HELLER, The mechanism of water diuresis in normal rats and rabbits as analyzed by inulin and diodone clearances. *J. Physiol. (Lond.)* 1945. *103*. 449–460.

FRIEDMAN, M., The creatinine, inulin and hippurate clearance in the rat. *Amer. J. Physiol.* 1947. *148*. 387–391.

FRIEDMAN, S. M. and C. A. LIVINGSTONE, The estimation of renal function in the rat by the use of diodrast and inulin. *Amer. J. Physiol.* 1942. *137*. 564–569.

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HERMAN, A. H., R. RIDDIOUGH, D. SMALL and R. EGDAHL, The effects of surgery on bile secretion and composition. *Surg. Forum* 1971. 22. 378–380.

LAMBERT, R., *Surgery of the digestive system in the rat.* Charles Thomas Publisher, Springfield, Illinois, U.S.A. 1965.

LEVINE, H. Q., C. WITMER and H. M. VARS, Interruption of the enterohepatic circulation and its effect on rat bile. *Amer. J. Physiol.* 1959. *197*. 1330–1332.

NILSSON, H. T., C. G. A. PERSSON, K. PERSSON, K. TEGNÉR and Å. RYRFELDT. The metabolism of terbutaline in dog and rat. *Xenobiotica* 1973. *3*. 615–621.

RIEGELMAN, S., J. C. K. LOO and M. ROWLAND, Shortcomings in pharmacokinetic analysis by conceiving the body to exhibit properties of a single compartment. *J. pharm. Sci.* 1968. *57*. 117–123.

ROWLAND, M., Application of clearance concepts to some literature data on drug metabolism in the isolated perfused liver preparation and in vivo. *Europ. J. Pharmacol.* 1972. *17*. 352–356.

SCHANKER, L. S. Secretion of organic compounds. In: Handbook of physiology. *Alimentary canal* 1968. Sect. 6. Vol. 5. Chapt. 4. pp. 2433–2449.

SCHANKER, L. S. and C. A. HOGBEN, Biliary excretion of inulin, sucrose and mannitol, analysis of bile formation. *Amer. J. Physiol.* 1961. *200*. 1087–1090.

WEEKS, J. R. and J. A. JONES, Routine direct measurement of arterial pressure in unanesthetized rats. *Proc. Soc. exp. Biol.* (N.Y.) 1960. *104*. 646–648.

ZYL, A., Thyroid function in relation to bile lipids and bile acids. *S. Afr. med. J.* 1959. 33. 618.

TABLE I Bile flow (ml min^{-1} kg^{-1}) and biliary clearance (ml min^{-1} kg^{-1}) of total radioactivity and unchanged drug after intraarterial injection of H inulin (0.2 μmol kg^{-1}) or ^{3}H terbutaline (1.8 μmol kg^{-1}) to unanesthetized male bile fistula rats. The values given are means ± S.D (N = 4–5).

Time (min)	Control	Inulin		Terbutaline		
	Bile flow	Bile flow	Clearance	Bile flow	Clearance (total)	Clearance (unchanged)
−60– 0	0.11±0.01	0.11±0.03	—	0.12±0.03	—	—
0– 20	0.12±0.02	0.12±0.04	—	0.13±0.03	—	—
20– 40	0.11±0.03	0.10±0.02	0.03	0.14±0.06	2.51	0.17
40– 60	0.13±0.02	0.10±0.02	0.04	0.13±0.05	4.24	0.98
60– 80	0.11±0.02	0.12±0.03	0.04	0.13±0.04	4.24	0.73
80–100	0.11±0.03	0.10±0.03	0.04	0.14±0.05	1.67	0.69
100–120	0.09±0.02	0.07±0.03	0.03	0.11±0.03	0.79	0.29
120–140	0.07±0.01	0.10±0.01	0.03	0.10±0.03	0.42	0.13
140–160	0.08±0.01	0.09±0.0_	0.03	0.09±0.01	1.34	0.16
160–180	0.07±0.01	0.08±0.02	0.03	0.07±0.02	0.38	0.1

The control rats were treated exactly as the dosed rats as regard to bile and blood sampling.

urine collecting periods) and Friedman (1947) (1.1–4.7 ml min kg obtained in rats anesthetized partly during urine collecting periods for blood sampling).

The distribution volume (Vdβ) of inulin was 0.25 ml g which is about the same as the relative extracellular water content of the body. The uptake into blood cells was low (Table II) which is a well known fact.

With terbutaline a dose of 1.8 μmol kg^{-1} (0.5 mg kg) was used which is a dose of pronounced pharmacodynamic activity. The bile to plasma concentration ratio of unchanged terbutaline varied from 1.2 to 7.5 and the biliary clearance from 0.12 to 0.98 ml min^{-1} kg (2 to 5 times the bile flow), indicating a concentrative transfer of terbutaline from plasma to bile. At physiological pH values, terbutaline is in the cationic form and it is known that several cations are actively secreted into the bile (Schanker 1968). The biliary clearance of total radioactivity ranged from 0.38 to 4.36 ml min kg. This indicates that the formed metabolite(s) is excreted more extensively than the unchanged drug. The cumulative excretion of radioactivity was larger than 0.3 of the administered dose of radioactivity. This is in agreement with values obtained by Nilsson *et al* (1973) in experiments with unanesthetized rats with interrupted enterohepatic circulation.

The plasma clearance of unchanged terbutaline was 5.2 ml min kg^{-1} which reflects excretion (biliary and urinary) as well as biotransformation of terbutaline.

The erythrocyte to plasma concentration ratio for terbutaline was about one. This indicates that the substance can either penetrate the membrane of red blood cells or attach to their surfaces. The distribution volume (Vdβ) of terbutaline was 0.58 ml g^{-1} which is about the same as the relative water content of the body. Autoradiographic studies have shown that terbutaline is not concentrated in any specific tissue or organ with the exception of liver and kidney (Bodin *et al* 1972).

Results obtained in the present investigation agree mainly with those obtained by other investigators in experiments with ed rats. As has been shown by *e.g.*

TABLE II. Some pharmacokinetic parameters for inulin (0.2 μmol kg^{-1}) and terbutaline (1.8 μmol kg^{-1}) administered intra-arterially to unanaesthetized male bile fistula rats. A two compartment open model has been used to characterize the pharmacokinetic profiles of the compounds.

Parameter	Inulin	Terbutaline
A (μmol ml^{-1})	0.62	34.5
B (μmol ml^{-1})	0.18	0.27
α (h^{-1})	7.22	6.42
β (h^{-1})	0.54	0.56
Vd_{β} (ml g^{-1})	0.25	0.58
Plasma clearance (ml min^{-1} kg^{-1})	2.3	5.2
Bile clearance (unchanged) (ml min^{-1} kg^{-1})	0.03–0.04	0.12–0.98
Erythrocyte/plasma ratio	0.1	1.2

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References

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From the Institute of Physiology University of Oslo, Norway

The Permeability of Fish Gills with Comments on the Osmotic Behaviour of Cellular Membranes

By

JOHAN B. STEEN[1] and SVERRE STRAY PEDERSEN

Received 9 January 1975

Abstract

STEEN, J B and S STRAY PEDERSEN *The permeability of fish gills with comments on the osmotic behaviour of cellular membranes* Acta physiol. scand. 1975 95 6–20

The permeability of eel gills to ions, lipid-soluble and -insoluble substances has been measured under steady-state conditions. The results obtained support the multi-pathway model for transcapillary transport (Stray-Pedersen and Steen 1975). The gills are 25 times more permeable to THO than to Na^+ or K^+ Metabolic inhibitors do not affect the transfer of substances across the gills. Exposure to EDTA or MS-222 changes the permeability properties to resemble those of an aqueous barrier The gills are 10 times less water permeable than the capillaries of the *rete mirabile*

Present concepts on the mechanisms of capillary wall penetration are largely based on informations obtained in experiments on perfused organs, particularily on mammalian skeletal muscles. The permeability constants in these experiments have been calculated on *the basis of studies of osmotic transients or from different types of clearance studies during* transient conditions (Crone 1970)

We wanted to test some of these concepts on simpler systems in which the phenomena of vessel wall penetration could be studied more directly and during steady-state conditions. Such studies have been performed in the *rete mirabile* of the eel (Stray-Pedersen and Steen 1975 Stray Pedersen 1975) These investigations showed that the mechanisms of transcapillary diffusion are more complex than generally assumed. As an alternative to the single-pore theory (Pappenheimer *et al* 1951 Pappenheimer 1953) a multipathway theory for diffusion across capillary membranes was suggested (Stray-Pedersen and Steen 1975)

The present paper reports experiments carried out on the gills of the eel Fish gills are specialized for gas exchange between blood and water The internal and external media are exposed to each other across a large, thin membrane made up by the walls of the gill lamellae. These are thin, flat, hollow sheets which are perfused by blood and irrigated by

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water. In the eel the lamellar wall is 2 to 5 μm thick and consists of a layer of flattened endothelial cells and two or more layers of epithelial cells. Between the endothelium and the epithelium various amounts of interstitial tissue are imposed. The intercellular clefts between the endothelial cells have tight junctions (Hughes and Grimstone 1965) similar to the capillaries of the brain of mammals (Brightman and Reese 1969). Direct determinations of the ionic composition of the gill tissue have not been performed. However we have measured the concentrations of Na^+, K^+ and Cl^- in squeezed gill filaments disintegrated by sulphuric acid (Stray-Pedersen and Nicolaysen, 1975) and found values of about 20, 60 and 80 meq/l respectively.

We are aware of the fact that the structure and function of the gill membrane are more complex than the capillary membrane of the *rete mirabile*. The aim of this investigations, however, was not primarily to describe the function of the normal gill as such, but rather to use the gill as a convenient preparation for permeability studies and thereby obtain informations about the permeability properties of its capillary membranes. In this preparation, as was also the case in the *rete*-preparation, the permeability of a substance could be determined directly from measurements of its concentrations on both sides of the capillary membrane during steady-state conditions. Since the maintenance of normal capillary permeability characteristics have been shown to be dependant on Ca^{++} and Mg^{++} (Chambers and Zweifach 1940, Wilbrandt *et al.* 1956, Nicolaysen 1971 a, b, Stray-Pedersen 1975), the effects due to removal of these ions were studied. The cells of the gill membrane are known to be metabolically very active, the organ playing an important role in for instance the osmoregulation (Maetz 1971, Motais and Garcia-Romeu 1972). It was therefore of great interest to study the effects of metabolic inhibitors, temperature and the drug MS-222 which was used as an anaesthetic in the present experiments. The results obtained were compared to those obtained in the *rete*.

Methods

Animals

The eels were exclusively silver eels adapted to fresh water, weighing between 100–200 g.

Surgery

The eel was made unconscious by a blow on the head and fastened in a holder (Steen 1963). A longitudinal opening exposed the ventral aorta which was cannulated for perfusion and tied off just anterior to where it branches off to the two posterior gill arches. The eel was turned with its dorsal side up and the spinal cord was removed to expose the dorsal aorta. This was cannulated and ligated so that only the two posterior arches were available for perfusion. Blood vessels to the brain and facial region arise from the two anterior gill arches only and no branching occur from the part of the gill circulation which was perfused in these experiments.

Ventilation

The gills were ventilated with water through a tube fastened into the mouth of the eel. The water was delivered either directly from the tap or by recirculation by an all plastic pump. The rate of ventilation had to be above 100 ml/min to assure adequate irrigation of all parts of the gills.

Perfusion

The gills were perfused with heparinized horse plasma prepared from fresh horse blood containing 20 I.U. heparin per ml. The plasma was stored at 20°C and used within 90 days.

TABLE I The ionic composition and osmolarity of horse plasma and eel plasma.

	K^+ mM/l	Na^+ mM/l	Cl mM/l	Ca^{++} mM/l	Mg^{++} mM/l	Osmola (mOsm
Horse plasma	3.3–3 6	125–135	95–102	2 2.5	1 1.2	270–290
Eel plasma	3 1–3.5	149–167	75–105	2.5–2.7	1 1 1	280–310

During perfusion with horse plasma the preparation was apparently very well maintained for s hours. Although the circulatory pathway had no leakages, lymphatic leakage did bother us initially tion of 1 mg adrenalin per 100 ml perfusate efficiently prevented this. It also caused the perfus circulate the lamellae and not the lymphatic shunts of the gills. (Steen and Kruysse 1965).

The composition of horse plasma has been compared to that of eel plasma in Table I. The prepa was perfused at 0.1 ml/min and at very low pressure (less than 5 cm H_2O). The outflow was collect known periods of time and weighed. Under normal conditions inflow and outflow volumes mea within 2% of each other

The normal cardiac output, and therefore also the total gill blood flow in eels of this size, is 1 to min When our preparation was perfused at these rates, the exchange of many substances was to to be detected with accuracy A perfusion rate of 0.1 ml/min was found to be optimal for our pur At this rate the exchange loss of substances across the gills was never less than one half of that obs at 1 ml/min. This shows that such a reduction of flow reduced the exchange area by at most 50 .

At the selected rate of perfusion and dead-space volume of the tubes, steady state conditions established within 30 min after the onset of perfusion. It could be shown experimentally that the t of the preparation were saturated with the isotopes (except for ^{42}K) in the course of this period.

T st substances

As test-substances were used urea ^{14}C, sucrose ^{14}C, dextran-75 000- ^{14}C (NEC 218B, MW 60 90 000), ethanol- ^{14}C, antipyrine- ^{14}C, tritiated water (THO), ^{42}K $^{24}Na^+$ Na and Cl The subst under study were usually added to the perfusate, but in some experiments they were added to the ven

Some relevant physico-chemical data of the test-substances are listed in T ble II

Analytical methods

Na^+ and K^+ was measured by standard flame photometry (Eppendorf). At concentrations within the r of normal plasma concentrations the measurements were accurate t within $\pm 2\%$

Cl was determined with n accuracy of $\pm 1\%$ on chloridemeter (electrical titration) (Eel).

The isotopes were counted in a Packard liquid scintillation counter Precautions were taken in to count the isotopes separately The input activities chosen were high enough to give relatively counting errors. *Additional inaccuracies by dilution gave values which were accurate within* ± 3 .

The osmolarity of samples was measured with an accuracy of ± 2 mOsm with an osmometer (Ad Digimatic mod 3 D).

The measurement of exchange

The exchange of a substance across the gill barrier was estimated from its concentrations in the perf t the output and input sides. This method is valid only if

1) The substance does not accumulate in the gill tissue.
2) The perfusate is not diluted due to osmosis or to admixture with lymph during perfusion.

Ad 1) The possible occurrence of tissue accumulation of test-substances was investigated by compa the amount of *substance appeari g in the ventilate* with that disappearing *from the perfusate*. No a mulation could be detected for any of the substances tested except for ^{42}K

Ad 2) D lution and lymph admixture was checked by comparing the concentration of dextran 75 to which the gill membrane was shown to be impermeable, n the perfusate at the input nd output s Since no difference was observed, no dilution could have taken place

THO was used as test-molecule in all experiments and consequently served a reference. The exch of any other substance was related to the THO-exchange measured simultaneously

TABLE II. Relevant physico-chemical data of the test-substances[a]

Substance	MW	Molecular (Ionic) radius (Å)	Olive oil/water partition co-efficient	$D_{20°C}$ 10^{-5} cm^2 sec^{-1}	D_x/D_{THO}
THO	20	1.5	0 0007	2.44	1
^{24}Na (Na^+)	24	—	Insoluble	1.48	0.61
Cl^-	35	—	Insoluble	1.92	0.79
^{42}K	42	—	Insoluble	1.92	0.79
Ethanol	46	2.4	0 022	1.24	0.51
Urea	60	2.6	0.00015	1.38	0.57
Antipyrine	188	4.1	0.032	0.68	0.28
Sucrose	342	5 6	Insoluble	0.52	0.21
Dextran-75 000	60 000–90 000	60	Insoluble	0 036	0.015

[a] For details see Bitty-Pedersen and Steen 1975.

Equations

The permeability (P) and diffusion (D) constants were calculated on the basis of the values obtained [of] the loss of substance during the passage through the gills. Since the ventilation flow (water flow) [i]s more than 1000 times at least to the perfusion flow the concentrations of test-substances in the [out]side were assumed to be constant. For the time in which there is a net diffusion from the perfusate [to] water of test-substance, having concentration in water of zero and the concentrations C_i and C_o in [the] input and output plasma solution, we get

$$C_o = C_i \quad ^{kt_i} \tag{1}$$

$$k = \frac{\ln (C_i/C_o)}{t_i} \tag{2}$$

[If] we assume that the concentration falls exponentially during the time t_i it takes the perfusate to pass [th]rough the gills. The mean concentration of the substance in the perfusate (= mean concentration differ[en]ce across the gill membrane), $\overline{\Delta C}$, may be found by integration of Eq. (1).

$$\overline{\Delta C} \; t_i = \int_{t=0}^{t=t_i} C \quad ^{kt} dt \tag{3}$$

$$\Delta C \; t_i \; k = C_i - C_i e^{-kt_i} \tag{4}$$

[Su]bstitution from Eqs. 1 and 2 into Eq. 4 gives:

$$\overline{\Delta C} = \frac{C_i \quad C_o}{\ln (C_i/C_o)} \tag{5}$$

[Ac]cording to Fick law of diffusion:

$$\dot{Q} = D\frac{\Delta CA}{l} \tag{6}$$

[where $\dot{Q}$ is] the amount of substance transported per unit time. A the area available to exchange, l the thickness [of] the gill barrier, D the diffusion coefficient
$\dot{Q}$ [is] also equal to:

$$\dot{Q} = (C_i - C_o) \, w \tag{7}$$

[i]n which w [is] the flow of perfusate.
The diffusion constant or the permeability constant is obtained by combining Eqs. 5, 6 and 7

$$D = \frac{Wl}{A} \ln \frac{C_i}{C} \tag{8}$$

or

$$P = \frac{W}{A} \ln \frac{C_1}{C} \qquad (9)$$

The units are $W = cm^3 sec^{-1}$, $l = cm$ and $A = cm^2$ whereas any units can be used for C_1 and C_0 as long as the same units are used in nominator and denominator. D has the units of $cm^2 sec^{-1}$ and P of $cm sec^{-1}$. The result corresponds to the equation derived by Crone (1963).

The values for the structural parameters l and A are difficult to estimate. The area of the lamellae of the two posterior gill arches was measured on eels of the same weight range as the eels used in the experiments by the methods of Hughes (1966). However, we found no good way to assess the proportion of lamellae actually perfused in each experiment. By using the anatomical values for A and l, 200 cm^2 and $5 \cdot 10^{-4}$ cm respectively, D and P are therefore most likely underestimated. In most cases we expressed the permeability by the factor $\ln (C_I/C_O)$, which we termed the permeability factor.

Accuracy of permeability values

The accuracy of $\ln (C_I/C_O)$ depends on the difference between C_O and C_I. This means that the accuracy will be higher, the higher the permeability. Generally the difference in concentration between input and output must exceed 3% of the input value to yield a permeability value of reasonable accuracy. Since the exchange of the ions and the lipid-insoluble test-molecules was of this magnitude, several samples were required in each experiment to obtain reliable values.

Results

Perfusions with normal plasma

In more than 100 expts. the gill preparation was perfused with horse plasma containing THO and one or more of the test-substances. Each experiment lasted for 2 to 7 h. The results obtained in a typical experiment are shown in Fig. 1. As seen from the figure there was a tendency for the loss of substances from the perfusate to be reduced with time. This tendency was most pronounced for ^{42}K. Except for this ion, the mutual relationship between the exchange of the different substances was maintained throughout an experiment. No differences between the osmolarity of the input and the output perfusates were obtained in these experiments.

The comparatively great loss of ^{42}K from the perfusate, however, was resulted in an accumulation of this isotope in the gill tissue rather than to flux across the gill barrier. This fact became evident when the loss of ^{42}K from the perfusate was compared to the gain in the ventilate. Since very high activities of the isotope were required, only 4 expts. of this type were performed. It turned out that about 10% of the amount of isotope lost from the plasma was recovered in the ventilate. We also counted the ^{42}K-activity in weighed pieces of gill tissue after about 3 h of perfusion with the K-activity of the perfusate. The specific activity of the tissue was found to be about 12 times as great as that of the perfusate. The total tissue activity agreed to within 15% to the total amount of K lost during the preceding perfusion.

TABLE III. The range of the loss of test-substances from the perfusate (expressed in per cent of input concentration) obtained in about 100 expts. (t 20–22°C, flow 0.1 ml/min, perfusate: modified horse plasma. The concentration of test-substance in the ventilat was zero.)

Substance	THO	Antipyrine	Ethanol	Urea	Sucrose	^{42}K	Na	Cl	D-75 000
% disappeared	35–80	10–40	30–75	10–15	0	10–30	2–4	2–4	0

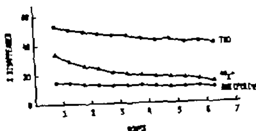


Fig. 1. Results from an experiment showing the typical change in the extraction of test-substances (expressed in per cent of the corresponding input concentration) during continuous perfusion with artificial plasma. Note the pronounced reduction in the loss of ^{42}K compared to that of THO and antipyrine during the perfusion. In this experiment the gills were irrigated with tap water (t = 7°C).

In Table III the percent disappearance from the perfusate of the different test-substances (except for ^{42}K) is quoted. The values represent the ranges obtained in about 100 expts. The differences in the exchange obtained in the experiments was most probably due to differences in the exchange area available, since the mutual relationship between the exchange of different substances showed far less variation. Therefore, the diffusion constants of the gill membrane quoted in Table IV were based on data from the experiments showing the highest exchange, i.e. where the exchange area was closest to the anatomical area. In the same table the relative diffusion anatomical area. In the same table the relative diffusion coefficients, D_s/D_{THO}, are given and compared to those obtained in the *rete*. Comparison with Table II shows that the permeability properties are very different from those of an aqueous barrier.

The exchange of the substances across the gill membrane in the direction from ventilate to perfusate was also investigated. To this end the ventilate containing the test-substances were recirculated and the concentrations recorded in the perfusate outflow. The exchange of THO, urea and ^{22}Na was the same as in the perfusate-to-ventilate direction, whereas the exchange of ^{42}K was found to be 10 times smaller.

Table IV. Diffusion constants and diffusion ratios of the gill membrane compared to those of the rete mirabile

	Gills Diffusion constants (20°C) D_s 10^6 cm^2 sec^{-1}	Gills D_s/D_{THO}	Rete[a] D_s 10^6 cm^2 sec^{-1}	Rete D_s/D_{THO}
THO	7 3	1	66 4	1
^{42}K	—	—	1.84	0.027
^{22}Na (Na^+)	0.3	0 04	1 44	0 022
Cl^-	0.3	<0 04	—	—
Urea	1.1	0 15	1 74	0.026
Sucrose	—	—	*0 58*	*0.009*
Ethanol	7 3	1 0	68.0	1.03
Antipyrine	3 6	0 5	57.2	0.86

[a] Assuming thickness of the retial capillary barrier of 2 μ. Data from Bray-Pedersen and Steen (1975).

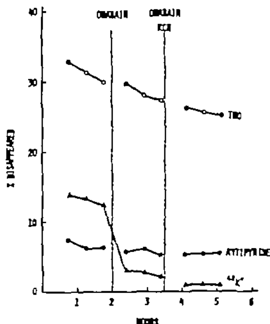


Fig. 2. The effect of ouabain and KCN. The extraction of THO and antipyrine (expressed as percent of the corresponding input concentration) did not change significantly during the experiment compared to that observed during normal conditions (see Fig. 1). However a marked reduction in the loss of $^{42}K^+$ from the perfusate was observed when plasma containing 2 mM/l of ouabain was introduced. The loss of ^{42}K appeared to be further reduced when 5 mM/l of KCN was added to the perfusate. In this experiment the gills were irrigated with tap water (t = 7°C).

Effect of metabolic inhibitors

The effects of the metabolic inhibitors, KCN and ouabain (strophantine), on the exchange across the gill membrane were studied in 6 experiments. After two hours of normal perfusion we switched to a perfusate containing 5 mM/l of KCN and/or 2 mM/l of ouabain. The results from one typical experiment are shown in Fig. 2.

As may be seen in the figure the introduction of ouabain (and KCN) lead to a marked reduction in the disappearance of K whereas the disappearance of the other test substances continued unchanged When the exposure to the inhibitors had lasted for about 30 min the disappearance of K^+ corresponded to that of ^{24}Na and Cl

Addition of KCN and/or ouabain to the ventilate did not lead to any changes in the exchange across the gill barrier of K or of any other substance under study

Effect of temperature

In 18 expts. the temperature of the ventilate was changed from 5°C to 20°C every other hour during a perfusion At each temperature the permeability factor $\ln(C_i/C_o)$, was calculated for the test-substances.

The effect of temperature was expressed as the change of the permeability factor caused by a 10°C change in temperature. This permeability change was called the Q_{10}. In Table V the Q_{10}-values obtained for the different test-substances are quoted and compared to the Q_{10}-values of the corresponding free diffusion coefficients in water

It is clear that Q for ions are similar to the Q of free diffusion while the Q for water and particularly for more lipid soluble substances is higher

Experiments at varying temperature in the presence of EDTA will be described in the next section.

Table V Q_{10}-values for the permeabilities of the test-substances obtained during perfusions with normal plasma and with plasma containing EDTA compared to the Q_{10}-values for the corresponding free diffusion coefficients.

	THO	Antipyrine	Ethanol	^{24}Na	^{42}K	Cl	Urea
Normal plasma	2.1	3.1	2.1	1.4	1.5	—	—
Plasma + 4 mM/l EDTA	1.4	1.7	1.3	1.3	1.5	1.2	1.1
Temperature coefficients for the free diffusion coefficients	1.35	1.33	1.35	1.35	1.35	1.35	1.35

The effect of removal of Ca^{++} and Mg^{++}

Calcium and magnesium ions are known to be of importance for maintenance of normal permeability and cell adhesivity. The effects due to removal of these ions were tested in experiments by adding the chelating agent, EDTA (as Na_2-EDTA), to the perfusate. EDTA was added in concentrations which corresponded to 120% of the total content of Ca and Mg in plasma. The results obtained in a typical experiment are shown in Fig. 3. EDTA did not affect the exchange of THO, ethanol or antipyrine. The exchange of ^{42}K was apparently reduced, whereas the exchange of ^{22}Na, Cl, urea and sucrose was dramatically increased. After 2 h of an EDTA-perfusion the ratios between the permeabilities of the test-substances had become strikingly similar to those between the free diffusion coefficients (Table VI).

Attempts to reverse the EDTA-effects were performed in many of these experiments. It turned out that if normal plasma perfusates were reintroduced after an EDTA-exposure of 15 min or less, normal permeability properties could be restored.

As described earlier the activity of ^{42}K in the gill tissue was 12 times higher than in the perfusate after 3 h of perfusion. When these experiments were continued for one hour with a perfusate containing sufficient EDTA to chelate all Ca^{++} and Mg^{++} the tissue activity of ^{42}K was found to be 7 times higher than the perfusate activity. When after 3 hours of perfusion with normal plasma a perfusate containing the same amounts of EDTA but with 2 mM/l ouabain in addition, the tissue activity was found to be only 2 times higher than

Table VI D_x/D_{THO}-ratios obtained in gills treated with EDTA and MS-222 compared to the D_x/D_{THO}-ratios of normal gills and of water (25°C).

	Gills			Water (i.e. free diffusion)
	EDTA	MS-222	Normal plasma	
THO	1.0	1.0	1.0	1.0
Na	0.55	0.43	0.04	0.61
Cl	0.67	0.53	0.04	0.79
Urea	0.63	0.5	0.15	0.57
Sucrose	0.33	—	—	0.21
Ethanol	—	—	1.0	0.51
Antipyrine	0.5		0.5	0.28

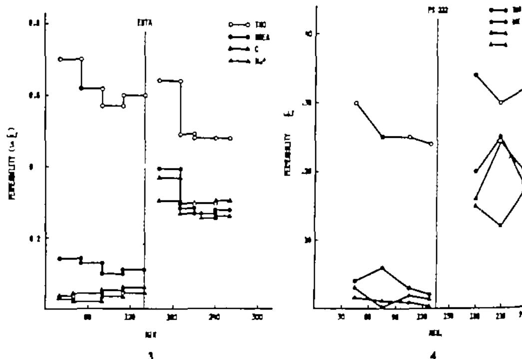


Fig. 3 The effect of EDTA. When EDTA was added to the perfusate in a concentration of about of the total content of Ca and Mg in plasma, the permeability of urea, Cl and Na+ increased tically The THO-permeability however appeared to remain fairly unchanged. In this experim gills were irrigated with water at 20°C.

Fig. 4 The effect of MS222. The addition of 2 g/l of MS222 to the ventilate lead to a marked in the permeability of urea, Cl and Na whereas the exchange of THO was not affected. In this ment the gills were rrigated with water at 20°C.

the perfusate activity In this latter case release of ^{42}K from the gill tissue was shown t place by the fact that the K -activity was temporarily about 50% higher in the c perfusate than in the input

The effect of temperature was also examined in gills treated with EDTA suffici give significant changes in permeabilities. As shown in Table V the Q for the test-subs (including THO and the lipid-soluble molecules) was very similar to that of the correspo free diffusion coefficients.

The effect of the local anaesthetic MS-222

MS-222 is an isomer of benzocaine (methanesulphonate of meta-aminobenzoic acid is widely used as an anesthetic for aquatic animals. It is added to water in ratios be 1 1 000 and 1 10 000

The effect of MS-222 (Sandoz) on the exchange was studied in 14 experiments. anaesthetic was added either to the ventilate or to the perfusate in concentrations of

During perfusions with plasma containing MS-222 no changes could be observed i exchange of the test-substances. When the drug had been added to the ventilate, how

milar to those obtained with EDTA (Fig. 4 and Table VI). In order to show that the effects bserved were not due to the low pH in the MS-222 ventilate, the gills were ventilated with water to which HCl had been added to match the pH-reduction caused by MS-222 (about pH 3.5). No effects could be observed in these perfusions (3 expts.). We also neutralized the water containing MS-222 by addition of NaOH (2 expts.) but the anaesthetic maintained its effects.

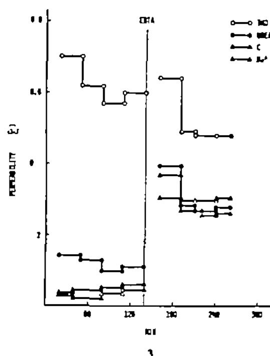
In some experiments MS-222 caused an initial increase in the THO-permeability (Fig. 4). It appeared that this effect occurred at different doses of MS-222 in different preparations.

The effects of MS-222 were reversible when the exposure lasted less than 30 min at 20°C.

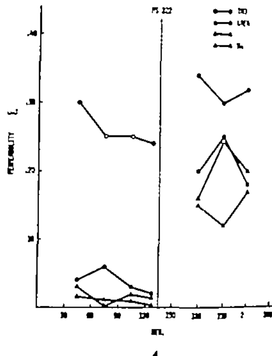
Discussion

The permeability studies performed on the *rete mirabile* (Stray-Pedersen and Steen 1975) indicated the existence of three different pathways available to transport across the capillary membrane: 1. One with lipoid character allowing the passage of lipid-soluble molecules, 2. "Water-pores" through which only water (or THO) may pass, and 3. Pores allowing the passage of hydrophilic molecules as well as of water. The latter pores were suggested to be equivalent to the Pappenheimer pores represented by the intercellular junctions, whereas the other two pathways are apparently confined to the endothelial cells proper. A transcellular passage of water has also been suggested by Alvarez and Yudilevich (1967) and by Faber and Hart (1967). The values obtained for the retial hydraulic conductivity indicated that the intercellular pores had diameters of about 1300 Å. Since the capillaries had a low permeability to albumin and dextran-75 000 (mol. diam. about 120 Å) the fibrillar matrix which may be observed within the junctions was assumed to behave as a *filter to these* molecules. Removal of Ca^{++} and Mg^{++} lead to a significant increase in the capillary permeability to lipid—insoluble molecules, whereas the exchange of THO and lipid-soluble molecules remained unchanged. The primary site of the action of Ca^{++} and Mg^{++} was suggested to be on the endothelial cell membrane and not on the pores (junctions). However after prolonged exposure to the chelating agent, EDTA, an increase in the permeability of high-molecular dextrans was observed. Since no changes in the junctional dimensions could be detected on electron micrographs from these *retia*, we assumed this permeability increase to dextrans to be due to changes in the structure of the fibrillar matrix of the junctions.

The present data show that the gill membrane have a permeability pattern which in many ways is similar to that of the retial capillaries. The gills were thus found to be 7 and 25 times more permeable to THO than to urea and ^{24}Na respectively (Table IV). This strongly indicates that the major part of the THO-diffusion and thereby the H_2O-diffusion takes place through the endothelial cells. Similarly lipid-soluble substances such as ethanol, antipyrine and partly urea must diffuse across the cell membranes since their permeabilities are significantly higher than would be predicted from their free diffusion coefficients as compared to those of Na and Cl. The Q_{10}-values of the permeabilities support this. Values similar to those of the free diffusion coefficients were found for ^{24}Na and Cl^- whereas lipid-soluble substances showed higher Q_{10}-values.



3



4

Fig. 3. The effect of EDTA. When EDTA was added to the perfusate in a concentration of about 120 of the total content of Ca and Mg in plasma, the permeability of urea, Cl and Na increased dramatically The THO-permeability however appeared to remain fairly unchanged. In this experiment the gills were irrigated with water at 20°C.

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the perfusate activity In this latter case release of K from the gill tissue was shown to take place by the fact that the K -activity was temporarily about 50% higher in the output perfusate than in the input

The effect of temperature was also examined in gills treated with EDTA sufficient to give significant changes in permeabilities. As shown in Table V the Q for the test-substances (including THO and the lipid-soluble molecules) was very similar to that of the corresponding free diffusion coefficients.

The effect of the local anaesthetic MS-222

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The effect of MS-222 (Sandoz) on the exchange was studied in 14 experiments. The anaesthetic was added either to the ventilate or to the perfusate in concentrations of 2 g/l.

During perfusions with plasma containing MS-222 no changes could be observed in the exchange of the test-substances. When the drug had been added to the ventilate, however the effects on the permeability properties of the gill membrane were rather dramatic and

It is interesting to compare the permeability properties of the gills with those of the capillaries in the *rete* of the swimbladder. The *rete* is an internal exchange organ designed to allow rapid gas exchange between arterial and venous blood. The gills should likewise allow rapid gas exchange but at the same time limit exchange of water and ions. Such comparison shows that the *rete* is 10 times more permeable to K^+ and THO than is the gill, whereas the urea-permeability is about the same in these organs (Stray-Pedersen and Steen 1975).

The diffusion coefficient of THO through the gill membrane is 7.3×10^{-8} cm sec^{-1}. Extrapolation to the size of water gives $D_{H_2O} = 8 \times 10^{-9}$ cm^2 sec^{-1}. This value is about 3 000 times smaller than the free diffusion coefficient for H_2O ($= 2.7 \times 10^{-5}$ at 25°C).

The proportion of the total gill area available for ion diffusion is very small. The free diffusion coefficient of Na^+ in water is 1.48×10^{-5} cm sec^{-1} whereas the D_{Na^+} for the gills is 2.9×10^{-11}. Provided Na^+ will diffuse only through intercellular pores and without restriction then only about 1:200 000 of the gill area is available to ion diffusion. Since the area of the gills perfused in these experiments was about 200 cm^2 the total slit area is roughly 0.001 cm^2.

In our experiments an osmotic water flow could not be detected. Therefore data are not available to calculate the pore size based on Poiseuille's equation. However since sucrose (mol. diam. = 11 Å) appears to be greatly restricted, the pore diameter is most likely not much greater than 20 Å. In this case there would be a total of 7×10^9 pores, *i.e.* 3×10^7 pores per square centimeter of gill surface.

The fact that the gill barrier has slits of small dimensions has consequences for its osmotic behaviour. It has been appreciated for many years that the osmotic flow of water can be hundreds of times faster than the diffusional flow of water if the separating membrane has pores with diameter above 50 Å. (Pappenheimer 1953, Mehta and Setnikar 1958.) However the larger the pore the smaller will be the osmotic pressure exerted by small molecules. It appears that the slit width of the gills may be the optimal compromise between the highest possible resistance to flow and the lowest possible osmotic effect of the smallest and most abundant ions. Although further research on flow through small pores are needed, we would like to stress that the flux of water across a biological barrier must be based on data on the hydraulic conductivity as well as on the effective osmotic pressure.

The work of Durbin (1960) on osmotic flow through cellulose membranes with varying pore diameter illustrates our point. Durbin used dialysis tubing and wet gel membranes with pore diameter of 23 and 82 Å respectively. The total pore area for the two membranes were the same and the hydraulic conductivity (L_p) was measured to be 25 and 370 µl/24 atm/mm respectively.

He measured the flow of water across the membranes when they separated water from solutions of different solutes. Using the hydraulic conductivity he could then calculate the effective osmotic pressure difference in each case. He found that the effective osmotic pressure was progressively smaller than calculated from the van't Hoff equation the smaller the ion. This deviation is expressed by the Staverman factor (σ) and expresses the "leakyness" of the membrane to each solute. Let us consider the osmotic behaviour when these membranes separate plasma from water. To simplify the discussion we assume plasma to consist of 280 meq/l "ions", 7 mM/l glucose and 1 mM/l of "proteins". Based on Durbin's

The size of the intercellular pores, must be considerably smaller in the gill membra than in the rete, since $^{22}Na^+$ was found to be at least 20 times as permeable as sucrose where the ratio of their free diffusion coefficients is 3 This would signal pore diameters below 20. This means either that the junctions of gill membrane are actually narrower than those the retial capillary membrane or simply reflect differences in the structure of the po matrix. Since the osmotic (or hydrodynamic) permeability of water across the gill membra was not measured, this point can not be ascertained.

The exchange of the test substances was the same in the direction from perfusate to ven late as in the opposite direction. The transbranchial transport was not influenced by t presence of metabolic inhibitors such as KCN or ouabain. However clear evidence f active uptake by the gill tissue of K^+ from the perfusate was obtained. According to Mae (1971) active transport may represent 20% of the total *influx* of NaCl. Our methods we not sufficiently accurate to detect such a difference between the exchange of Na or C from ventilate to perfusate and the exchange from perfusate to ventilate.

The accumulation of K in the gill tissue could be effectively abolished by ouaba and/or KCN but no effect on its transbranchial exchange could be detected.

The divalent ions, Ca^{++} and/or Mg^{++} play an important role in maintaining the selectiv permeability property of the fish gill barrier (Potts and Fleming 1970, Cuthbert and Mae 1971). When these ions were removed by EDTA in the present study the permeabili pattern of the gills changed dramatically and in a similar way as was observed in the re (Stray-Pedersen 1975) The permeabilities of ions and lipid-insoluble molecules increase by a factor of 5 to 20, whereas the permeabilities of water and lipid-soluble molecules we not affected to an extent which could be detected with certainty After 30 min of EDTA exposure the permeability pattern but not the absolute permeabilities of the gills resemble closely that of an aqueous barrier (Table VI) The permeability ratio between Cl and sucro obtained at such circumstances were about 2, the ratio between their free diffusion coefficien being about 4 The fact that dextran-75 000 did not pass the EDTA treated gills to an measurable extent indicated that severe edema or tissue damage had not occurred.

The main site of action of Ca^{++}/Mg^{++} resides most likely in the cell membranes rathe than in the intercellular junctions. Removal of these ions is known to cause permeabili changes in a variety of cells (Morill and Robbins 1967). If the effect of EDTA was due to widening of the pores to such an extent as to give a 20 fold increase in the Na -permeability then a measurable increase in the permeability of THO should be expected. This was no found The fact that the accumulation of K was significantly smaller in the EDTA treated gills, indicated the cells to be more leaky to ions. Moreover in the EDTA-treate gills the Q -values of the permeabilities of the test-substances were almost similar to thos of the corresponding free diffusion coefficients (Table VI), indicating that removal of Ca Mg^{++} lead to alterations in the lipid character of the membrane.

The local anaesthetic, MS 222 elicited the same type of permeability changes as did EDTA It is therefore reasonable to suggest the site of action of this drug also to be on the ce membranes. Since the permeability changes could only be induced when MS 222 had bee added to the ventilate it is tempting to suggest that its "receptor" sites are on the epitheli cell rather than on the endothelial cell

uptake in a 100 g eel in freshwater has been measured to be about 1 µl/min (Evans 1969). The total pore area is 1/200 000 of the gill area, but the diffusion coefficient for water through gills is 1/3 000 of that through the pores. The diffusion of water across the pores will therefore be 1.1 × 3 000/200 000 = 0.014 µl/min, i.e. only 1.5% of the total water diffusion occurs through the pores.

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References

Al-Awqati, Q. A. and D. L. Yudilevich, Capillary permeability and tissue exchange of water and electrolytes in the stomach. *Amer. J. Physiol.* 1967. *213*. 315–322.

Bennett, M. W. and T. S. Reese, Junctions between closely apposed cell membranes in the vertebrate brain. *J. cell. Biol.* 1969. *40*. 648–677.

Chambers, R. and B. W. Zweifach, Capillary endothelial cement in relation to permeability. *J. cell. comp. Physiol.* 1940. *15*. 255–272.

Crone, C., The permeability of capillaries in various organs as determined by use of the indicator diffusion method. *Acta physiol. scand.* 1963. *58*. 292–305.

Crone, C., Capillary permeability—techniques and problems. In: *Capillary Permeability*. Ed.: C. Crone and N. A. Lassen. Munksgaard, Copenhagen. 1970. 15–31.

Cuthbert, A. W. and J. Maetz, The effect of calcium and magnesium ions on sodium fluxes through gills of *Carassius auratus L.* *J. Physiol.* (Lond.) 1972. *221*. 633–643.

Durbin, R. P. Osmotic flow of water across permeable cellulose membranes. *J. gen. Physiol.* 1960. *44*. 315–326.

Evans, D. H. Studies on the permeability to water of selected marine, freshwater and euryhaline teleosts. *J. exp. Biol.* 1969. *50*. 689–703.

Faber, J. J. and F. M. Hart, Transfer of charged and uncharged molecules in the placenta of the rabbit. *Amer. J. Physiol.* 1967. *213*. 890–894.

Hughes, G. M. The dimensions of fish gills in relation to their function. *J. exp. Biol.* 1966. *45*. 177–195.

Hughes, G. M. and A. V. Grimstone, The fine structure of the secondary lamellae of the gills of *Gadus pollachius*. *Quart. J. micr. Sci.* 1965. *106*. 343–353.

Maetz, J. Fish Gills, Mechanisms of salt transfer in fresh water and sea water. *Phil. Trans. B*, 1971. *262*. 209–249.

Meschia, G. and I. Setnikar, Experimental study of osmosis through collodion membranes. *J. gen. Physiol.* 1958. *42*. 429–444.

Morrill, G. A. and E. Robbins, The role of calcium in the regulation of the steady-state levels of sodium and potassium in the HeLa cell. *J. gen. Physiol.* 1967. *50*. 781–792.

Motais, R. and F. Garcia-Romeu, Transport mechanisms in the teleostean gill and amphibian skin. *Amer. Rev. Physiol.* 1972. *34*. 141–176.

Nicolaysen, G., Intravascular concentrations of calcium and magnesium ions and edema formation in isolated lungs. *Acta physiol. scand.* 1971 a. *81*. 325–339.

Nicolaysen, G. Increase in capillary filtration rate resulting from reduction in the intravascular calcium ion-concentration. *Acta physiol. scand.* 1971 b. *81*. 517–527.

Pappenheimer, J. R., Passage of molecules through capillary walls. *Physiol. Rev.* 1953. *33*. 387–423.

Pappenheimer, J. R., E. M. Renkin and L. M. Borrero, Filtration, diffusion and molecular sieving through peripheral capillary membranes. A contribution to the pore theory of capillary permeability. *Amer. J. Physiol.* 1951. *167*. 13–46.

Potts, W. T. W. and W. R. Fleming, The effects of prolactin and divalent cations on the permeability to water of *Fundulus kansae*. *J. exp. Biol.* 1970. *53*. 317–327.

Steen, J. B. The physiology of the swimbladder in the eel *Anguilla vulgaris*. II. The reabsorption of gases. *Acta physiol. scand.* 1963. *58*. 138–149.

TABLE VII Staverman factor osmotic pressure and osmotic flow for two synthetic membranes separati plasma from water (See text for details.)

Concentration in "plasma	Staverman factor (σ)		Osmotic pressure (π) $\pi = \sigma \ \Delta C \ 24 \ 10^{-4}$ (atm)				Osmotic flow (V) $V = \pi \ L_p$ (μl/min)	
	Dialysis tubing	Wet gel	Dialysis tubing		Wet gel		Dialysis tubing	Wet gel
280 meq/l ions	0.01	0.002	0.067		0.0134			
7 mM/l glucose	0.20	0 016	0.034	0.125	0.003	0 035	0.125	0 5
1 mM/l protein	1 0	0.73	0.04		0.0184			

data on the Staverman factor for different substances we assess reasonable values for eac of these three substance categories (Table VII) We can now calculate the osmotic pressur exerted and the resulting water flow for each of the three components across both mem branes The osmotic pressure is

$$\pi = \Delta C \ \sigma \ 24 \quad 10^{-4} \text{ atm}$$

since a ΔC of 1 mM/l causes an osmotic pressure of 24 10^{-4} atm. Table VII compares th calculated values. We shall notice that while van t Hoffs equation predicts plasma (ΔC 300 mOsm/l) to exert an osmotic pressure of 7.2 atm, the porosity reduces this to 0.125 for the dialysis tubing and 0 035 atm for the wet gel.

This osmotic pressure will act to such water across the gill barrier and into the plasma The capillary pressure will act in the opposite direction The mean branchial blood pressur in the eel is about 40 cm H O whereas the osmotic pressure across the two cellulose mem branes of Durbin was 126 and 35 cm H_2O respectively This suggests that if the intercellula pores of the gill really have widths of about 20 Å, then the blood pressure in the gill capil laries may be of *significance* in reducing the osmotic water uptake in *freshwater fishes*.

Finally we can estimate the proportion of the total water diffusion which occurs through the pores.

The total diffusional water flux across the gills is equal to

$$\dot{Q} = D \frac{A \Delta C}{l}$$

Where

$\dot{Q}$ = net diffusion of water from water to plasma in cm /min.
D = the diffusion coefficient = 8 0 10^{-6} cm /sec = 4.8 × 10⁻ cm /min
A = gill area = 200 cm
ΔC = difference in water concentration between plasma and ventilation water
= 300 mM/l = 0.3 mM/cm
l = thickness of barrier = 5μ = 5 10^{-4} cm

Using these values $\dot{Q}$ becomes 5 10^{-4} mM/min. Since 1 mM of water occupies 18 μl the total rate of water diffusion becomes 0 9 μl/min This indicates that osmotic water uptake may not be more important than diffusional water uptake of water since the total water

uptake in a 100 g eel in freshwater has been measured to be about 1 µl/min (Evans 1969). The total pore area is 1/200 000 of the gill area, but the diffusion coefficient for water through gills is 1/3 000 of that through the pores. The diffusion of water across the pores will therefore be 1·1·3 000/200 000 = 0.014 µl/min, i.e. only 1.5% of the total water diffusion occurs through the pores.

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References

Al Askari, O. A. and D. L. Yudilevich, Capillary permeability and tissue exchange of water and electrolytes in the stomach. *Amer. J. Physiol.* 1967. *213*. 315–322.

Brightman, M. W. and T. S. Reese, Junctions between intimately apposed cell membranes in the vertebrate brain. *J. cell. Biol.* 1969. *40*. 648–677.

Chambers, R. and B. W. Zweifach, Capillary endothelial cement in relation to permeability. *J. cell. comp. Physiol.* 1940. *15*. 255–272.

Crone, C. The permeability of capillaries in various organs as determined by use of the "indicator diffusion" method. *Acta physiol. scand.* 1963. *58*. 292–305.

Crone, C., Capillary permeability—techniques and problems. In: *Capillary Permeability*. Ed. C. Crone and N. A. Lassen. Munksgaard, Copenhagen. 1970. 15–31.

Cuthbert, A. W. and J. Maetz, The effect of calcium and magnesium ions on sodium fluxes through gills of *Carassius auratus* L. *J. Physiol.* (Lond.) 1972. *221*. 633–643.

Durbin, R. P. Osmotic flow of water across permeable cellulose membranes. *J. gen. Physiol.* 1960. *44*. 315–326.

Evans, D. H., Studies on the permeability to water of selected marine, freshwater and euryhaline teleosts. *J. exp. Biol.* 1969. *50*. 689–703.

Faber, J. J. and F. M. Hart, Transfer of charged and uncharged molecules in the placenta of the rabbit. *Amer. J. Physiol.* 1967. *213*. 890–894.

Hughes, G. M., The dimensions of fish gills in relation to their function. *J. exp. Biol.* 1966. *45*. 177–195.

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Maetz, J. Fish Gills, Mechanisms of salt transfer in fresh water and sea water. *Phil. Trans. B*, 1971. 262. 209–249.

Mauro, O. and I. Sivinskar, Experimental study of osmosis through a collodion membrane. *J. gen. Physiol.* 1958. *42*. 429–444.

Morrill, G. A. and E. Robbins, The role of calcium in the regulation of the steady-state levels of sodium and potassium in the HeLa cell. *J. gen. Physiol.* 1967. 50. 781–792.

Motais, R. and F. Garcia-Romeu, Transport mechanisms in the teleostean gill and amphibian skin. *Amer. Rev. Physiol.* 1972. *34*. 141–176.

Nicolaysen, G., Intravascular concentrations of calcium and magnesium ions and edema formation in isolated lungs. *Acta physiol. scand.* 1971 a. *81*. 325–339.

Nicolaysen, G. Increase in capillary filtration rate resulting from reduction in the intravascular calcium ion-concentration. *Acta physiol. scand.* 1971 b. *81*. 517–527.

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Potts, W. T. W. and W. R. Fleming, The effects of prolactin and divalent cations on the permeability to water of *Fundulus kansae*. *J. exp. Biol.* 1970. 53. 317–327.

Steen, J. B. The physiology of the swimbladder in the eel, *Anguilla vulgaris*. II. The reabsorption of gases. *Acta physiol. scand.* 1963. 33. 138–149.

TABLE I Effect of 60 min cold exposure in hydropenic man

	GFR ml/min 1 73 m²			RPF ml/min × 1 73 m²			C_{osm}/C_{In} %			C_{Na}/C_{In} %		
	a	b	c	a	b	c	a	b	c	a	b	c
Mean	107	111	106	603	533	561	2.39	2.67	2.85	1 11	1.38	1.56
S.D	19 35	27 39	26.91	163.5	143 7	191.8	0.47	0.55	0.48	0.29	0.43	0 43
p		>0.05	>0.05		>0 05	>0.05		<0.05	<0.01		<0.05	<0 01

Effect on glomerular filtration rate, renal plasma flow fractional excretion of osmoles, fractional excretion of sodium. Individual data, means and standard deviations from 9 healthy subjects. a = means of 3 30-min control periods at room temperature b = 0–30 min in cold c = 30–60 min i cold p – values are calculated by paired observations.

The hydropenic state was effected by the withdrawal of all liquid and solid nourishment for 19 h prior to the study Hydropenic state was checked i the laboratory by 3 consecutive 30 min urine samples which had to show stable flow and an osmolality of > 1 000 mOsm/kg H_2O

Standard clearance technique was used. Glomerular filtration rate (GFR) was measured as the clearance of inulin and renal plasma flow (RPF) as the clearance of PAH, both in ml/min and 1 73 m² body surface 0.5 ml/kg of a solution consisting of 85 mg/ml of inulin (Laevosan Gesellschaft) and 30 mg/ml of para-aminohippuric acid PAH (Merck, Sharp & Dohme) was given as a prime dose. The solution was then infused by a motor-driven syringe at a constant rate of 0.5 ml/min. The clearance infusion was allowed to equilibrate for 90 min.

The bladder was catheterized with a double lumen catheter and emptied t the end of each period by air insufflation, vacuum suction and gentle suprapubic compression. To ensure a complete emptying, the bladder was rinsed with 20 ml of sterile water at the end of each period.

Mid period blood samples were drawn from a cubital vein. Arterial systolic blood pressure was recorded manually from one arm by mercury manometer

The volunteers were naked and covered with blankets. They spent three control periods of 30 min at room temperature. Then the blankets were removed and the temperature on the table was lowered to + 15°C. All experiments were terminated by two periods of 30 min in cold.

Standard statistical methods were used including pa red observations and Student s t test. All alues are given as means ± standard deviation.

Analyses

In blood and urine samples, sodium was analyzed n flame photometer (IL Model 143) and the osmolality was analyzed cryoscopically in Knauer osmometer Inul was nalyzed accordi g to Heyrovsky (1956) and PAH according to Brun (1951)

T bular reabsorption of solute free w ter ($T^c_{H_2O}$) *was calculated from the expression* ($C_{osm} - V$), here V = rine flow in ml/mi and C_{osm} = clearance of osmoles in ml/min.

Results

Individual data from 9 hydropenic subjects are listed in Table I and II

Effect on renal hemodynamics

Exposure to cold for 1 h at + 15°C did not influence glomerular filtration rate At room temperature, mean GFR was 107 ± 19 4 ml/min and 1 73 m body surface and after 60 min of cold 106 ± 26.9 ml/min and 1 73 m body surface (p > 0 05) (Table I)

During cold exposure renal plasma flow changed from 603 ± 163 5 ml/min and 1 73 m² body surface at room temperature to 561 ± 191 8 ml/min and 1 73 m body surface but this change did not differ statistically from controls (p > 0 05) (Table I)

TABLE II. Effect of 60 minutes cold exposure in hydropenic man

	Arterial BP mm Hg			$T^c_{H_2O}/C_{In}$ %			U_V/C_{In} %		
		b			b			b	
Mean	116	131	133	1.68	1.89	2.00	0.72	0.78	0.85
S D	9 0	11 6	10 0	0.31	0 33	0 32	0 2	0 2	0 2
P		0 001	<0.001		<0.05	<0 01		>0 05	0 <0 05

Effect on arterial blood pressure, free water reabsorption and urinary flow For details see Table I

Effect on renal excretion of osmoles and sodium

The fractional excretion of osmoles (C_{osm}/C_{In}) increased significantly after cold exposure as did the fractional sodium excretion (C_{Na}/C_{In}). Mean osmolal excretion increased from 2.39 ± 0 47 to 2.85 ± 0.48 per cent (p < 0.01) and mean sodium excretion from 1 11 ± 0.29 to 1.56 ± 0 43 per cent (p 0.01) (Table I).

Effect on blood pressure free water reabsorption and urinary flow

Arterial blood pressure rose during cold from a mean of 116 ± 9 mm Hg at room temperature to 131 ± 11 6 after 30 min in cold and to 133 ± 10 mm Hg after 60 min in cold. The increase was significant in both periods (p < 0.001) (Table II).

Free water reabsorption, correlated to glomerular filtration rate ($T^c_{H_2O}/C_{In}$), increased significantly during cold exposure from a mean at room temperature of 1.68 ± 0.31 to 2.00 ± 0.32 per cent (p 0.01) (Table II).

Already after 30 min in cold, there was a small, but significant rise in $T^c_{H_2O}$. Fig. 1 shows the relationship between the rise in free water reabsorption and the rise in excretion of osmoles during 60 min of cold exposure.

In the hydropenic state, mean urinary flow was rather small and stable during control periods and cold exposure At room temperature, mean urinary flow correlated to the filtration (U_V/C_{In}) amounted to 0.72 ± 0.2 per cent. It showed a slight, but significant increase to 0 85 0.2 per cent after 60 min in cold (p 0 05) (Table II).

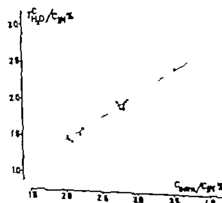


Fig 1 Relationship between free water reabsorption ($T^c_{H_2O}$) and excretion of osmoles (C_{osm}) both correlated to the filtration rate in 9 hydropenic subjects during 60 min cold exposure. Each subject is represented by 3 dots, one in each of 3 control periods at room temperature, one after 30 min in cold and one after 60 min in cold. The regression line is expressed by the equation y 0.08 0 67 (p 0 001).

Discussion

The present results include the finding that cold exposure in man caused an increased urinary excretion of osmoles. Since this excretion was not accounted for by changes in GFR and since the load of osmoles was not changed during the study the increased excretion of osmoles was most likely due to a reduced net tubular reabsorption.

Sodium is considered to be reabsorbed without water in the ascending limb of the loop of Henle. This creates a hypertonic medullary interstitium which in the presence of ADH contributes to the concentration of the final urine by reabsorbing water from the collecting ducts (Pitts 1970) The more sodium actively pumped out of the ascending limb of the loop the higher osmotic gradient will move water from the collecting ducts. Thus, in hydropenia free water reabsorption, $T^{c}_{H_2O}$, can be used as an index of sodium reabsorption by the ascending limb of the loop of Henle.

Increased $T^{c}_{H_2O}$ in connection with increasing clearance of osmoles is shown by several authors. Goldberg *et al* (1965) reported that during hypertonic saline diuresis in hydropenic man, $T^{c}_{H_2O}$ increased progressively even when osmolal clearance was exceeding 27 ml/min and 1 73 m^2 body surface. During mannitol diuresis however $T^{c}_{H_2O}$ was shown to rise to an upper limit above which the curve levelled off This took place when the clearance of osmoles exceeded 12 ml/min and 1 73 m body surface. In hydropenic dogs, undergoing hypertonic mannitol diuresis, Earley *et al* (1961) found this maximum in $T^{c}_{H_2O}$ to occur at an osmolal clearance of 12–15 ml/min.

In a previous report from this laboratory it was suggested that tubular reabsorption of sodium during cold was related to increases in arterial blood pressure (Wallenberg and Granberg 1974). Aperia *et al.* (1971) when increasing the clearance of osmoles as a function of renal artery perfusion pressure in hydropenic dogs, found the maximum in $T^{c}_{H_2O}$ to correspond to an osmolal clearance of 7 ml/min/100 ml GFR, representing a perfusion pressure of more than 180 mm Hg. In the present study there was a significant linear relationship between free water reabsorption and clearance of osmoles. Clearance of osmoles however did not exceed 4 ml/min/100 ml GFR the arterial pressure did not exceed 150 mm Hg and the curve did not show any declination

In previous studies performed in water diuresis, we did not find any measurable increase in urinary flow during cold exposure (Atterhög *et al* 1975 Wallenberg and Granberg 1974). The present results showed a small, but significant increase in urinary flow after 60 min in cold If this represents a true difference in hydropenic state compared to water diuresis is an open question

Previous findings indicated that the reabsorption of sodium by the distal tubules was important for the cold-induced excretion of sodium in water loaded man (Wallenberg 1974). The excretion of sodium was shown to be inhibited by infusion of hyperoncotic albumin solution during cold exposure. Parallel to this inhibition, the albumin infusion correspondingly improved fractional distal reabsorption of sodium. This result was in accordance with the view that a cold-induced disturbance in the balance between hydrostatic and oncotic pressures in peritubular capillaries might have negatively affected sodium reabsorption in the diluting segment of distal tubules.

In the present study the results showed a small, but significant increase in free water reabsorption during cold, despite a significant rise in arterial perfusion pressure. It is presumed that reduction in tubular reabsorption of sodium is dependent of increases in arterial pressure in the kidney. Obviously this is not true for the ascending limb of the loop of Henle within the small range of pressure rise produced by cold. This is supported by the findings of Aperia *et al.* (1971) showing no reduction in $T^c_{H_2O}$ in hydropenic dogs until a perfusion pressure in the kidney above 180 mm Hg.

The parallel increase in osmolal clearance and in free water reabsorption would speak in favour to an increased concentration of osmoles entering Henle's loop during cold. The results does not exclude the increased $T^c_{H_2O}$ to be due to a reduced reabsorption of sodium by the proximal tubules. However with the methods used in this study it is not possible to directly evaluate the effect of cold exposure on different sites of the nephron.

References

Aperia, A. C., C. G. O. Broberger and S. Söderlund, Relationship between renal artery perfusion pressure and tubular sodium reabsorption. *Amer. J. Physiol.* 1971. 220. 1205–1212.

Atterhög, J. H., P. Carlens, P. O. Granberg and L. R. Wallenberg, Cardiovascular and renal responses to acute cold exposure in waterloaded man. *Scand. J. clin. Lab. Invest.* 1975. 35. In press.

Braun, C., A rapid method for the determination of para-aminohippuric acid in kidney function tests. *J. Lab. clin. Med.* 1951. 37. 955–958.

Earley, L. E., M. Kahn and J. Orloff, The effects of infusions of chlorothiazide on urinary dilution and concentration in the dog. *J. clin. Invest.* 1961. 40. 857–866.

Earley, L. E., J. A. Martino and R. M. Friedler, Factors affecting sodium reabsorption by the proximal tubule as determined during blockade of distal sodium reabsorption. *J. clin. Invest.* 1966. 45. 1668–1684.

Goldberg, M., D. K. McCurdy and M. A. Ramirez, Differences between saline and mannitol diuresis in hydropenic man. *J. clin. Invest.* 1965. 44. 182–192.

Heyrovsky, A., A new method for the determination of inulin in plasma and urine. *Clin. Chim. Acta.* 1956. 1. 470–474.

Pitts, R. F., Physiology of the kidneys and body fluids. *Year Book Med. Publ., Chicago.* 1970. Sec. Ed. Chapt. 7.

Wallenberg, L. R., Reduction in cold-induced natriuresis following hyperoncotic albumin infusion in man undergoing water diuresis. *Scand. J. clin. Lab. Invest.* 1974. 34. 233–239.

Wallenberg, L. R. and P. O. Granberg, The relationship between cold-induced natriuresis and arterial blood pressure in man. *Scand. J. clin. Lab. Invest.* 1974. 34. 225–231.

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Potentiation by Prostaglandins E_1, E_2, and $F_{2\alpha}$ of the Contraction Response to Transmural Stimulation in the Bovine Iris Sphincter Muscle

By

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Abstract

GUSTAFSSON, L. P HEDQVIST and H LAGERCRANTZ. *Potentiation by prostaglandins E_1, E_2 and $F_{2\alpha}$ of the contraction response to transmural stimulation in the bovine iris sphincter muscle* Acta physiol scand. 1975 95 26–33

The contraction response to transmural stimulation of the bovine iris sphincter muscle was abolished by atropine but was left virtually unchanged by hexamethonium, and by adrenergic neuron and receptor blockers, indicating activation of postganglionic cholinergic nerve fibres. Low doses of prostaglandins E_1, E_2 and $F_{2\alpha}$ (0.1–5.0 ng/ml) consistently and apparently in a dose-dependent manner enhanced the contraction response to transmural stimulation. Prostaglandins E_1 and E_2 were equipotent while $F_{2\alpha}$ was at least 10 times less active. Within the same dose range the prostaglandins also enhanced the contraction response to exogenous acetylcholine, indicating that part of the enhancing effect of the prostaglandins on neuromuscular transmission was due to a postjunctional action. A spontaneous increase in tone of the preparation commonly occurred about 1 h after the beginning of the experiment. SC 19220, a potent antagonist of prostaglandin action, reduced the tone and the contraction responses as well as the enhancing effect of the prostaglandins. It is concluded that prostaglandins might serve the function of controlling neuroeffector transmission and muscular tone in the sphincter muscle of the bovine iris.

Prostaglandins of the E and F series (PGE, PGF) seem to be present in the iris of the rabbit, cat, sheep and cattle (Änggård and Samuelsson 1964 Ambache *et al* 1966, Ambache and Brummer 1968, Posner 1970). The iris contains the enzyme necessary for conversion of eicosatrienoic acid into PGs (van Dorp *et al* 1967) Mechanical irritation of the eye causes increased formation and release of PGs, as does electrical stimulation of the iris (Ambache *et al* 1965 Posner 1970, 1973). Irritation of the eye causes miosis, increased intraocular pressure and increased protein content of the aqueous humour effects which can be mimicked by local application of PGs (Waitzman and King 1967 Beitch and Eakins 1969).

PGs are potent inhibitors of sympathetic neuroeffector transmission and locally formed PGs have been postulated to negatively feed-back control the release of adrenergic transmitter (Hedqvist 1970, 1973) PGEs have also been claimed to influence parasympathetic

uroeffector transmission since PGE_1 inhibits the effect of vagal nerve stimulation in the bbit heart (Wennmalm and Hedqvist 1971). Similarly PGE_1 inhibits gastric secretion duced by vagal stimulation in the rat (Shaw and Ramwell 1968). On the other hand, rt of the stimulant action of PGE on the guinea pig ileum as well as that of $PGF_{2\alpha}$ on liva secretion from canine submandibular glands have been suggested to be mediated via tivation of cholinergic fibres (Horton 1965, Hahn and Patil 1972, 1974).

In the present paper are reported some actions of PGs on contraction response to parasympathetic nerve stimulation in the sphincter muscle of the bovine iris. A preliminary account of the results has been presented elsewhere (Gustafsson, Hedqvist and Lagercrantz 1973).

Methods

ovine eyes were enucleated 5–10 min post mortem, immediately placed in ice-cold Tyrode and used within 4 h. Two strips of the sphincter muscle, with an approximate length of 15 mm were dissected free from e upper and lower margins of the iris. The preparation was mounted in 5 ml organ bath and connected an isotonic smooth muscle transducer (Harvard Instr. Inc.) with load of 0.2–0.4 g. Changes in basal ne as well as induced contractions of the preparation were recorded on Grass model 5 Polygraph or Honeywell ardinate writer. Replacing the isotonic transducer by force displacement strain gauge transducer (Grass FT03) gave consistent recordings. As bath fluid was used either Tyrode (concentrations in M): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.3, glucose 5.5, or Krebs olution (concentrations in mM): NaCl 120, KCl 5.9, $CaCl_2$ 2.5, MgCl 1.2, $NaHCO_3$ 15.5, NaH_2PO 1.2, lucose 11.5. The bath fluid was kept at 37°C and was gassed with 6.5% CO_2 in O_2. The preparation was stimulated through platinum electrodes along the wall of the bath and a Grass S4 stimulator delivering rains of biphasic pulses (1–20 Hz, 1 ms duration, 15–25 V effective strength, 1–100 periods) at 1 min ntervals. A standard type of stimulation (3 Hz, 15 pulses) was routinely used unless otherwise stated.

The following compounds were used: acetylcholine chloride (SIGMA Chemicals, St Louis, U.S.A.), atropine sulfate (SIGMA), guanethidine sulfate (Ciba-Geigy, Basel, Switzerland), hexamethonium bromide (SIGMA), histamine chloride (ACO, Sweden), l-noradrenaline hydrochloride (SIGMA), phentolamine methanesulfonate (Ciba-Geigy), propranolol hydrochloride (ICI Ltd., Great Britain), prostaglandin E_1, E_2 and $F_{2\alpha}$ as free acids (kindly supplied by Dr J. Pike, The Upjohn Co, Kalamazoo, Mich., U.S.A.) and SC 19220 (1-acetyl-2(8-chloro-10,11-dihydrodibenz (b, f) (1.4) oxazepine-10-carbonyl) hydrazine), (kindly supplied by Dr J. H. Sanner, The G. D. Searle and Co, Chicago, Ill., U.S.A.).

Results

When the isolated bovine iris sphincter muscle was mounted in the organ bath, it remained at its resting length (15–17 mm) for approximately 1 h whereafter it slowly began to contract. This occurred independently of whether the preparation was stimulated or not. In preparations which reached a stable high tone the final length was 4–5 mm. Although the spontaneous increase in basal tone could be partially counteracted by repeated washing of the preparation, experiments were always carried out during the first hour after mounting the preparation unless otherwise stated.

Transmural stimulation (3 Hz, 15 pulses) of the sphincter muscle produced a rapid and marked contraction which in some preparations was followed by a poststimulatory relaxation below the resting level. Single pulses were also sufficient to induce a contraction. The contraction induced by transmural stimulation could be mimicked by acetylcholine (Ach) 0.4 µg/ml, and was completely abolished by atropine, 0.15 µg/ml. The relaxation compo-

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Abstract

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The contraction response to transmural stimulation in the bovine iris sphincter muscle was blocked by atropine but was left virtually unchanged by hexamethonium, and by adrenergic neuron and receptor blockers, indicating activation of postganglionic cholinergic nerve fibres. Low doses of prostaglandins E_1, E_2 and $F_{2\alpha}$ (0.1–5.0 ng/ml) consistently and apparently in a dose-dependent manner enhanced the contraction response to transmural stimulation. Prostaglandins E and E_2 were equipotent while ... was at least 10 times less active. Within the same dose range the prostaglandins also enhanced the contraction response to exogenous acetylcholine, indicating that part of the enhancing effect of the prostaglandins on neuromuscular transmission was due to a postjunctional action. A spontaneous increase in tone of the preparation commonly occurred about 1 h after the beginning of the experiment. SC 19220, a potent antagonist of prostaglandin action, reduced the tone and the contraction responses as well as the enhancing effect of the prostaglandins. It is concluded that prostaglandins might serve the function of controlling neuroeffector transmission and muscular tone in the sphincter muscle of the bovine iris.

Prostaglandins of the E and F series (PGE, PGF) seem to be present in the iris of the rabbit, cat, sheep and cattle (Änggård and Samuelsson 1964 Ambache *et al* 1966, Ambache and Brummer 1968 Posner 1970) The iris contains the enzyme necessary for conversion of eicosatrienoic acid into PGs (van Dorp *et al* 1967). Mechanical irritation of the eye causes increased formation and release of PGs, as does electrical stimulation of the iris (Ambache *et al* 1965 Posner 1970 1973) Irritation of the eye causes miosis, increased intraocular pressure and increased protein content of the aqueous humour effects which can be mimicked by local application of PGs (Waitzman and King 1967 Beitch and Eakins 1969).

PGs are potent inhibitors of sympathetic neuroeffector transmission and locally formed PGs have been postulated to negatively feed-back control the release of adrenergic transmitter (Hedqvist 1970, 1973) PGEs have also been claimed to influence parasympathetic

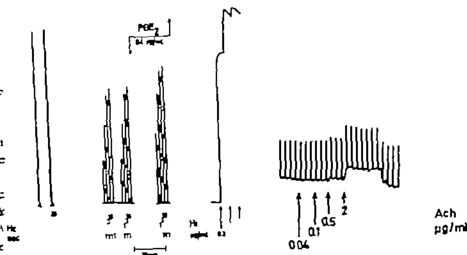


Fig. 3. Isolated bovine iris sphincter muscle. Submaximal contraction responses to 1 sec pulse trains at 3, 10, and 20 Hz shown to be potentiated by addition of PGE_2. To the left and right maximal amplitude of contraction responses to respectively transmural stimulation (20 Hz for 5 and 10) and histamine.

Fig. 4. Isolated bovine iris sphincter muscle. Effect of subspasmogenic and spasmogenic doses of acetylcholine on contraction responses to transmural stimulation (3 Hz 3 pulses, 1 ms) at 1 min intervals.

PGE_2 enhanced the contraction response to transmural stimulation at 1 3, 10, and 20 Hz, provided the stimulation period was kept short (1 s) (Fig. 3). When the preparation was stimulated at 20 Hz for 5 or 10 s a near maximal contraction occurred, as witnessed also by the contraction responses to histamine, and PGE_2 did not significantly alter the response. In Table I is summarized the effect of 2 doses of PGE_2 on the contraction response to transmural stimulation at different stimulation frequencies. It can be seen that, although PGE_2 significantly enhanced the contraction response at all frequencies used, the effect decreased with increasing stimulation frequency

In 13 other experiments it was investigated whether acetylcholine, as reported above for

TABLE I. Isolated bovine iris sphincter muscle. Contraction response to 1 periods of transmural stimulation at 1, 3, 10 and 20 Hz, 10 min after addition of PGE_2, compared to that obtained during pre-ceding corresponding control stimulation. number of experiments. Statistical analysis according to Student's t-test for paired and unpaired data ($= p < 0.05$, $= p < 0.01$).

	PGE_2 ng/ml	Stim freq.	Responses in of control		
			Mean	± S.E.	p <
7	0.2	1	154.4	10.2	.01
7	0.2	3	157.3	8.7	.01
7	0.2	10	151.4	6.6	.01
7	0.2	20	124.7	4.5	.01
8	0.4	1	179.0	14.3	.001
8	0.4	3	149.0	9.2	.01
8	0.4	10	131.6	5.0	.001
8	0.4	20	125.6	4.5	.001

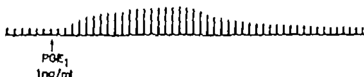


Fig. 1 Isolated bovine iris sphincter muscle. Effect of PGE_1 (1 ng/ml) on contraction response transmural stimulation (3 Hz, 15 pulses, 1 ms, supramaximal voltage) at 1 min intervals. Wash at dot

nent which was regularly observed after atropine administration could be blocked by guanethidine, 0 8 µg/ml or propranolol 20 µg/ml In these doses guanethidine or propranolol did not alter the contraction response to transmural stimulation. In a 10 times higher dose guanethidine produced an inhibition of induced contractions (*cf* Boura and Green 1965) Hexamethonium, 5–20 µg/ml, or phentolamine, 4 µg/ml, had no effect on contraction response to transmural stimulation. Administration of noradrenaline (NA), 0.2 µg/ml relaxed the preparation, an effect which was altered to contraction when propranolol 20 µg/ml had been given in advance.

Addition to the bath of PGE in doses ranging from 0.2–1 0 ng/ml consistently and dose dependently increased the contractile response to transmural stimulation (3 Hz, 15 pulses (Fig. 1). Lower doses (down to 1 pg/ml) did not alter responses while higher doses (0.5 ng/ml) caused a direct contraction of the preparation without further increasing the response to transmural stimulation. The potentiation by PGE_1 was visible within 60 s, reached a maximum after 5–10 min and remained at this level for at least another 10 min period. After washing the preparation the responses returned to normal over a period of 5–10 min.

PGE_2 produced qualitatively and quantitatively the same effects as PGE on the sphincter muscle, whereas $PGF_{2\alpha}$ was found to possess a much weaker stimulant effect on the contraction response to transmural stimulation. A summary of the effects of different doses of PGE PGE_2 and $PGF_{2\alpha}$ is given in Fig. 2.

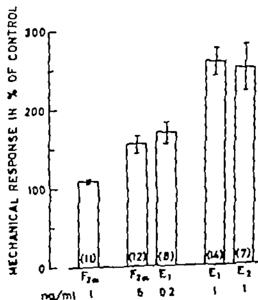


Fig. 2. Isolated bovine iris sphincter muscle. Comparison of enhancing effect on contraction response to transmural stimulation by PGE_1, PGE_2 and $PGF_{2\alpha}$. Stimulation parameters as in Fig. 1. Response measured immediately before (control) and 6 min after addition of respective prostaglandin. Mean values ± S.E. Figures within brackets = number of expts.

Ferreira, S. H., A. Herman and J. R. Vane, Prostaglandin generation maintains the smooth muscle tone of the rabbit isolated jejunum. *Brit. J. Pharmacol.* 1972, *44* 328–330.

Gustafsson, L. E., P. Hedqvist and H. Lagercrantz, Prostaglandin mediated enhancement of effector responses to cholinergic nerve stimulation. *Acta physiol. scand.* 1973, Suppl. 396, 106.

Hahn, R. A. and P. N. Patil, Salivation induced by prostaglandin $F_{2\alpha}$ and modification of the response by atropine and physostigmine. *Brit. J. Pharmacol.* 1972, *44* 527–533.

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Hedqvist, P. Studies of the effect of prostaglandins E_1 and E_2 on the sympathetic neuromuscular transmission in some animal tissues. *Acta physiol. scand.* 1970, *79* Suppl. 345, 1–40.

Hedqvist, P. Prostaglandin mediated control of sympathetic neuroeffector transmission. *Adv. Biosc.* 1973, *9* 461–473.

Horton, E. W. Biological activities of pure prostaglandins. *Experientia* (Basel) 1965, *21* 113–118.

Patil, P. N., Adrenergic receptors of the bovine iris sphincter. *J. Pharmacol. exp. Ther.* 1969 *166*, 299–307.

Posner, J. The release of prostaglandin E_2 from the bovine iris. *Brit. J. Pharmacol.* 1970, *40*, 163P 164P.

Posner, J. The action of prostaglandins on the bovine sphincter pupillae. *J. Physiol.* (Lond.) 1971 *217* 25P 26P.

Posner, J. Prostaglandin E_2 and the bovine sphincter pupillae. *Brit. J. Pharmacol.* 1973, *49* 415–427.

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Schaeppi, U. and W. P. Koella, Reaction of isolated pig iris sphincter to electrical stimulation and acetylcholine. *Amer. J. Physiol.* 1964 *206*, 255–261.

Schaeppi, U. and W. P. Koella, Adrenergic innervation of cat iris sphincter. *Amer. J. Physiol.* 1964 b, *207* 273–278.

Shaw, J. E. and P. W. Ramwell, Inhibition of gastric secretion in rats by prostaglandin E_1. In *Prostaglandins Symp. of the Worcester Found. for Experimental Biology*. Eds. P. W. Ramwell and J. E. Shaw. Interscience, N.Y. 1968, pp. 55–64.

Waitzman, M. B. and C. D. King, Prostaglandin influences on intraocular pressure and pupil size. *Amer. J. Physiol.* 1967 *212*, 329–334.

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Therefore, the potentiating effect of the PGs must be regarded an interaction with cholinergic neuroeffector transmission.

It should be noted that, although the PGs were tested over a wide range of doses, inhibition of the contraction response was never seen This is interesting from the point of view that PGs have been reported to inhibit effector responses to vagal nerve stimulation (Shaw and Ramwell 1968, Wennmalm and Hedqvist 1971), and indicates that the effect of PGs on cholinergically innervated tissues is not unitarily stimulant or inhibitory

The fact that the responses to acetylcholine were enhanced by the PGs indicated that the PGs exert at least part of their stimulant effect on neuroeffector transmission in the sphincter muscle postjunctionally However an additional prejunctional effect on acetylcholine release cannot be ruled out as has also been suggested for the stimulant action of $PGF_{2\alpha}$ on saliva secretion from the canine submandibular gland (Hahn and Patil 1974). Further studies on transmitter release from the nerve endings in the sphincter muscle are required to establish this possibility

The presence of PG in, and release from, the iris sphincter muscle has been documented (Änggård and Samuelsson 1964 Posner 1973). Moreover increased PG release seems to occur during periods of increased tone (Posner 1973), and a high tone can be effectively counteracted by PG synthesis inhibitors (Gustafsson *et al* 1973) or by PG effector blockade (Posner 1973 present results). These observations merit the assumption that locally formed PG modulates or even generates the intrinsic tone of the iris sphincter muscle as has been postulated for the smooth muscle of the gut (Ferreira *et al.* 1972). However inhibition of neurogenically elicited contractions by PG effector blockade and enhancement by exogenous PG in concentrations below those which increase the tone of the muscle invites the intriguing possibility that endogenous PG perhaps to an even greater extent, serves the function of controlling neuroeffector transmission in the sphincter muscle.

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References

AMBACHE, N and H C. BRUMMER, A simple chemical procedure for distinguishing E from F prostaglandins, with application to tissue extracts. *Brit J Pharmacol* 1968 *33* 16– 170

AMBACHE, N H C. BRUMMER, J G ROSE nd J WHITING, Thin-layer chromatography of spasmogenic unsaturated hydroxyacids from various tissues. *J Physiol.* (Lond.) 1966. *185* 77P 78P

AMBACHE, N L. KAVANAGH and J WHITING, Effect of mechanical stimulation on rabbits eyes release of active substance in anterior chamber perfusates. *J Ph siol.* (Lond.) 1965. *176* 378–408.

ÄNGGÅRD E. and B. SAMUELSSON, Smooth-muscle stimulating lipids in sheep iris. The identification of prostaglandin $F_{2\alpha}$. *Biochem. Pharmacol.* 1964 *13* 281 283

BEITCH B. R. and K. E. EAKINS, The effects of prostaglandins on the intraocular pressure of the rabbit. *Brit. J Pharmacol.* 1969 *37* 158–167

BERGSTRÖM, S., R. ELIASSON, U S. v EULER and J SJÖVALL, Some biological effects of t crystalline prostaglandin factors. *Acta ph siol. scand.* 1959 *45* 133 144

BOURA, A. L. A. and F A. GREEN, Adrenergic neurone blocking gents. *Ann. Rev Pharmacol.* 1965. *5.* 183– 1..

DORP D A. van, G. H JOUVENAZ and C. B. STRUIJK, The biosynthesis of prostaglandi in pig ey iris *Biochim bi phys. Acta* (Amst.) 1967 *137* 396.

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References

Ambache, N and H C. Brummer, A simple chemical procedure for distinguishing E from F prostaglandins, with application to tissue extracts. *Brit J Pharmacol* 1968 *33* 162 170.

Ambache, N H C. Brummer, J G. Rose and J Whiting, Thin-layer chromatography of spasmogenic unsaturated hydroxyacids from various tissues. *J Physiol.* (Lond) 1966. *185* 77P 78P

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Pulmonary flow and pressures, as well as pulmonary blood volume and extravascular lung water content were measured. Although large effects on vascular pressures were seen, only small changes were detected as regards fluid contents in the lung.

A preliminary report on some of the results has been given elsewhere (Karlsen, Aarseth and Bo 1973).

Methods

Cats weighing 2.3–5.2 kg were used. Anaesthesia was induced by ether inhalation. A catheter was then inserted into a femoral vein and chloralose (50 mg/kg b.w.) was given i.v. The animals were tracheostomized, curarized (Alloferin, Roche, 0.5 mg/kg b.w.), and given positive pressure ventilation by an Ideal Starling pump. The pump frequency was 21 strokes/min, and end expiratory pressure was kept at 2 cm H_2O. The tidal volume was regulated so as to keep pH of arterial blood between 7.35 and 7.45. At 30 min intervals a "deep breath" was given by briefly increasing the inflation pressure to 18–20 cm H_2O.

A midsternal incision permitted wide opening of thorax. Catheters were inserted into the pulmonary artery through the wall of the right ventricle and into the left atrium through its appendage. Catheters were also inserted into both femoral arteries. Intravascular pressures were recorded by Statham pressure transducers (P23D6 and P23Db) connected to a Grass multichannel recorder Model 7 B. An electromagnetic flowprobe mounted on the ascending aorta was connected to a flowmeter (Nycotron, Norway), and instantaneous as well as mean flow were continuously followed on the Grass recorder.

Silk snares were loosely placed around the roots of the two upper and the right middle lung lobe. These snares were later tightened one by one. Thereby the circulation through one particular lobe was arrested and the lobe could be removed for evaluation of its water and blood content. As these lobe parameters were calculated relative to the net dry tissue weight of the lobe, the values obtained for the 3 lobes could be compared. All lobe-isolations were performed at the end of an expiration and thus with a transpulmonary pressure of 2 cm H_2O.

When the initial surgical procedures had been finished, the animals were left undisturbed for 45 min. 3 ml blood had earlier been withdrawn, and the erythrocytes labelled with ^{51}Cr and washed thoroughly (Aarseth 1970). Now these erythrocytes were mixed with an equal amount of ^{125}I-labelled human serum albumin. A weighed sample of this mixture was injected into the animal, and 10 min later the snare was tightened around the root of the right upper lobe. Simultaneously arterial blood was sampled for hematocrit measurement and for analysis of its isotope content.

Up to this point of the procedure all animals were given the same handling. The second and third lobes, to be removed later, should however reflect changes brought about by catecholamines or hypoxic stimulation. Therefore one of these types of stimulation was initiated 8 min before the second lobectomy and maintained continuously for 28 min until the third lobe was removed. Thus the effects of catecholamine-infusion as well as of general hypoxia were studied after 8 as well as after 28 min of stimulus administration.

The following groups were established:

Control group: 5 animals where the 3 lobes were removed without any intervening procedures.

Adrenaline group: 7 animals receiving 5 µg/kg b.w./min of adrenaline.

Noradrenaline groups: (a) 4 animals receiving 5 µg/kg b.w./min of noradrenaline and (b) 4 other animals receiving 1.25 µg/kg b.w./min of noradrenaline.

Hypoxic group: 4 animals were ventilated with a gas mixture of air and nitrogen with a P_{O_2} of about 75 mm Hg.

P_{O_2} was measured in arterial blood samples from all animals throughout the experiments. For this a Radiometer oxygen electrode was used.

Lung blood and extravascular water content. The removed lobes were frozen in liquid nitrogen and thereafter cut so that the tissue could be divided into several test tubes. These tissue samples and also the blood samples taken were analyzed for their isotope content. Their wet weights were found, and they were then dried at 75 C until the weight became stable. From the radioactivity in blood and tissue, and from the wet and dry weights of the samples, the blood content and extravascular water content in the lobes were calculated relative to the net dry tissue weight of that lobe. These weight-related volumes could then be compared from lobe to lobe. In each animal the first lobe gave the reference values for that animal. The values for blood and water content in the second and third lobe were then expressed as per cent of these reference values. A more detailed description of the calculations has been given previously (Aarseth and Bo 1972).

Statistical calculations have been done by a two-sided Wilcoxon's two-sample test.

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From the Institute of Physiology University of Oslo, Norway

Effects of Catecholamine-Infusions and Hypoxia on Pulmonary Blood Volume and Extravascular Lung Water Content in Cats

By

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Abstract

Aarseth, P., J Karlsen and G Bo *Effects of catecholamine-infusions and hypoxia on pulmonary blood volume and extravascular lung water content in cats* Acta physiol. scand. 1975 95 34–40

The effects of catecholamine-infusions and of hypoxia on lung blood volume and on extravascular lung water content have been studied in anesthetized cats with opened chests. To this end a biopsy technique, with isotope labelling of blood and with successive removal of the smaller lung lobes, was employed. Mean pulmonary arterial pressure increased by 30–75% upon infusing catecholamines or upon inducing general hypoxia, the latter stimulus being the more powerful one. Pulmonary blood volume did not increase during these procedures, whereby an active constriction of pulmonary vessels thus apparently took place. The extravascular lung water content was found to be reduced in animals infused with noradrenaline or ventilated by hypoxic air whereas a small increase was observed in the animals receiving adrenaline. The difference might reflect domination of precapillary vasoconstriction in the former groups, whereas post capillary vasoconstriction could be more pronounced with adrenaline.

It has been suggested that increased lung water content with impairment of lung function develops upon elevation of the blood level of catecholamines and upon increased sympathetic nervous activity Such adrenergic effects are thought to be involved in the pulmonary edema induced by increased intracranial pressure (Bean and Beckman 1969) and a variety of neurogenic stimuli (Luisada 1967) Accidental injections of large amounts of adrenaline to humans can also give rise to an acute, fulminant pulmonary edema (Ersoz and Finestone 1971)

A wet lung syndrome seen as a consequence of traumatic and hypovolemic shock is also characterized by congestion and interstitial edema (Moore *et al* 1969) In experimental studies pulmonary effects of traumatic and hemorrhagic shock seemed to be prevented when hormonal release from the adrenal medulla was eliminated (Bo and Hognestead 1971)

In the present work we have attempted to evaluate the effects on the pulmonary vasculature of infused catecholamines, with an infusion rate comparable to maximal endogenous hormonal release In addition one group of animals was tested with general hypoxemia, which is again known to be a powerful stimulus for endogenous release of catecholamines.

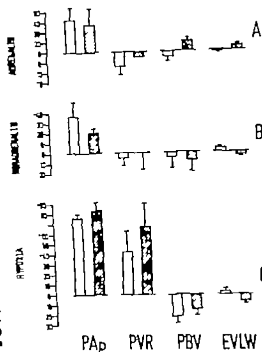


Fig 1 Development of various pulmonary parameters upon catecholamine infusions or generalized hypoxia. In cat lungs in situ, the arterial pressure (PA_p), the vascular resistance (PVR), the blood volume (PBV) and the extravascular water content (EVLW) were measured after either catecholamine infusion or ventilation hypoxia for 8 min and for 28 min respectively. The values given represent the percentage changes from control values obtained in each animal before stimulation. (A) adrenaline, 5 µg/kg b.w./min; (B) noradrenaline, 1.25 or 5 µg/kg b.w./min (C) hypoxia, arterial P_{O_2} = 24–29 mm Hg. Mean values ± S.E.

left atrial pressure increased during the adrenaline infusion, but the mean value after 28 min was only 8 mm Hg, and in only one animal did it exceed 10 mm Hg.

Effects of noradrenaline (*Fig 1 B*). There seemed to be no differences on the effects caused by noradrenaline given at the two rates selected, 1.25 µg/kg b.w./min and 5 µg/kg b.w./min. All animals given noradrenaline were therefore grouped together. Upon these infusions cardiac output and the pulmonary arterial pressure increased to the same extent by some 25 %. The pulmonary vascular resistance was thus unchanged during infusion of noradrenaline. The changes in lung blood content were small and inconsistent, whereas the extravascular lung water content showed a small decrease on the border of being significant (p = 0.07). After 28 min of noradrenaline-infusion there was also a tendency towards increased left atrial pressure, but the changes were small and not statistically significant.

Effects of general hypoxemia (*Fig 1 C*). The ventilating gas for the 4 animals in this group had an oxygen tension of about 75 mm Hg. The resulting arterial P_{O_2} was measured to be 24–29 mm Hg. Despite this grave hypoxemia the cardiac output increased and remained high throughout the experimental period. The pulmonary arterial pressure increased, however, much more markedly (by nearly 100 %) than did the cardiac output, indicating an increased pulmonary vascular resistance. Despite the tremendous increase in lung arterial pressure, the pulmonary blood content, when looking at the 2 removed lobes together, was

TABLE I The effects of lobectomies on circulatory parameters in remaining lung in cats. Three small lobes were removed one by one. The values for pulmonary vascular resistance pulmonary arterial pressure and cardiac output are the ones found immediately before the removal of the different lobes. Lung blood content and lung extravascular water are the values found in each lobe; calculated relative to the net dry tissue weight of that lobe. For the first lobe the absolute values are given, while for the second and third lobe the percentage change from the first lobe are given. Mean values ± S.D

	Lobe No 1 – 100 %	Lobe No. 2 %	Lobe No. 3 %
Pulmonary vascular resistance	0.039 ± 0.04 mm Hg ml^{-1} × min	118 ± 9	141 ± 9
Pulmonary arterial pressure	15.4 ± 4.3 mm Hg	102 ± 5	111 ± 3
Cardiac output	304 ± 100 ml/min	88 ± 3	81 ± 7
Lung blood content	4.4 ± 1.4 ml/g dry tissue weight	114 ± 14	1[illegible] ± 6
Lung extravascular water	4.69 ± 0.09 ml/g dry tissue weight	95 ± 2	100 ± 1

Results

The present method used for determination of lung fluid contents involved three successive lobectomies. These lobectomies might *per se* influence the parameters studied. Such influences could be reflected in the control group of 5 animals and the data from this group are presented in Table I. As the pulmonary vascular bed was reduced by the lobectomies, the lung vascular resistance increased from 0 039 mm Hg ml min initially to 0 052 mm Hg ml min, when the third lobe was removed The cardiac output decreased some 10-20% during the experimental period, while the mean pulmonary arterial pressure increased from 15 mm Hg to 17 mm Hg at the time of the third lobectomy The blood content increased by 14 and 22 % respectively in the second and third lobe, while the lung extravascular water content decreased by some 5 % in the second lobe and returned towards the initial level in thie third lobe.

When in the following we attempted to evaluate the effects of catecholamines and hypoxemia proper the above mentioned changes induced by the lobectomies *per se* were subtracted from the changes found in the various test groups of animals In Fig. 1 A–C are shown the *net* effects thus obtained.

All animals (except the ones given hypoxic air) had a normal arterial P_{O_2} level at the beginning of the experiment (mean value 98 mm Hg). All animals (except the ones given hypoxic air) also had an arterial blood P_{O_2} above 65 mm Hg (mean value 88 mm Hg) at the end of the experiment

Effects of adrenaline (*Fig 1 A*) Aortic flow increased by about 40 % when adrenaline was infused at a constant rate of 5 µg/kg b w./min. Even though this marked increase in cardiac output was not maintained throughout the 28 min of infusion, there was still an augmented cardiac flow at the end of the experiment (p = 0 05). The pulmonary arterial pressure increased by about 25 % (p = 0 01), but the pulmonary vascular resistance seemed to decrease as compared to the value seen in the control group The changes in blood content of the lung lobes were small and inconsistent The extravascular water content of the lobes was found to be unchanged after 8 min of infusion but there may have been a small increase in the third lobes when these were compared to those of the control group (p = 0 15). The

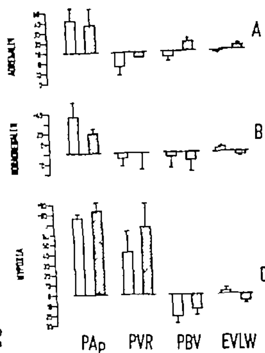


Fig. 1 Development of various pulmonary parameters upon catecholamine infusions or generalized hypoxia. In cat lungs in situ, the arterial pressure (PA_p), the vascular resistance (PVR), the blood volume (PBV) and the extravascular water content (EVLW) were estimated after either catecholamine infusion or ventilation hypoxia for 8 min and for 28 min respectively. The values given represent the percentage changes from control values obtained in each animal before stimulation (A) adrenaline, 5 μg/kg b.w./min, (B) noradrenaline, 1.25 or 5 μg/kg b.w./min, (C) hypoxia, arterial P_{O_2} 24–29 mm Hg. Mean values ± S.E.

left atrial pressure increased during the adrenaline infusion, but the mean value after 28 min was only 8 mm Hg, and in only one animal did it exceed 10 mm Hg.

Effects of noradrenaline (Fig. 1 B). There seemed to be no differences on the effects caused by noradrenaline given at the two rates selected, 1.25 μg/kg b.w./min and 5 μg/kg b.w./min. All animals given noradrenaline were therefore grouped together. Upon these infusions cardiac output and the pulmonary arterial pressure increased to the same extent by some 25%. The pulmonary vascular resistance was thus unchanged during infusion of noradrenaline. The changes in lung blood content were small and inconsistent, whereas the extravascular lung water content showed a small decrease on the border of being significant ($p = 0.07$). After 28 min of noradrenaline-infusion there was also a tendency towards increased left atrial pressure, but the changes were small and not statistically significant.

Effects of general hypoxemia (Fig. 1 C). The ventilating gas for the 4 animals in this group had an oxygen tension of about 75 mm Hg. The resulting arterial P_{O_2} was measured to be 24–29 mm Hg. Despite this grave hypoxemia the cardiac output increased and remained high throughout the experimental period. The pulmonary arterial pressure increased, however much more markedly (by nearly 100%) than did the cardiac output, indicating an increased pulmonary vascular resistance. Despite the tremendous increase in lung arterial pressure, the pulmonary blood content, when looking at the 2 removed lobes together, was

TABLE I The effects of lobectomies on circulatory parameters in remaining lung in cats. Three small lobes were removed one by one. The values for pulmonary vascular resistance, pulmonary artery pressure and cardiac output are the ones found immediately before the removal of the different lobes. Lung blood content and lung extravascular water are the values found in each lobe as calculated relative to the net dry tissue weight of that lobe. For the first lobe the absolute values are given, while for the second and third lobe the percentage change from the first lobe are given. Mean values ± S.D

	Lobe No. 1 = 100 %	Lobe No. 2 %	Lobe No. 3
Pulmonary vascular resistance	0 039 ± 0 04 mm Hg ml^{-1} × min	118 ± 9	141 ± 9
Pulmonary arterial pressure	15 4 ± 4.3 mm Hg	10. ± 5	111 ± 3
Cardiac output	304 ± 100 ml/min	88 ± 3	81 ± 7
Lung blood content	4 4 ± 1 4 ml/g dry tissue weight	114 ± 14	122 ± 6
Lung extravascular water	4 69 ± 0 09 ml/g dry tissue weight	95 ± 2	100 ± 1

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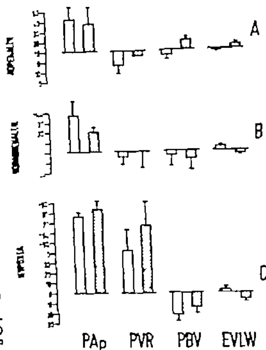


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Cardiac output	304 ± 100 ml/min	88 ± 3	81 ± 7
Lung blood content	4.4 ± 1.4 ml/g dry tissue weight	114 ± 14	122 ± 6
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1973) as well as in cat lungs in situ (Aarseth, Bø and Hauge 1975). The latter of these works demonstrated that a substantial increase in left atrial pressure (and thus also in pulmonary capillary pressure) was needed in order to increase lung water content some few per cent. Thus left atrial pressure had to be elevated to about 10 mm Hg in order to achieve a 3% increase in water content (Aarseth, Bø and Hauge 1975). Two thirds of such a pressure increase will probably extend to the pulmonary capillaries. In the present experiments with infusion of adrenalin, the pulmonary arterial pressure increased from about 12 to 18 mm Hg, and the left atrial pressure increased from about 5 to about 8 mm Hg during the infusion. If there is predomination of postcapillary constriction (reduced distensibility) and at the same time some precapillary vasodilatation (reduced resistance), these pressure changes might result in a capillary pressure increase of as much as 7 mm Hg. The findings of a 4% increase in extravascular lung water content would then be in good agreement with the above mentioned results of Aarseth, Bø and Hauge (1975).

The small reduction in lung extravascular water seen after noradrenaline infusion for 28 min, appears to indicate a predominant precapillary action of this drug. The lung arterial pressure increase was thus similar to that seen after infusion of adrenaline but the vascular resistance was not apparently reduced. Also, the pulmonary blood volume seemed to be smaller after noradrenaline infusion than after adrenaline, again indicating a more marked upstream location of the noradrenaline induced vasoconstriction.

The infusion rate of adrenaline used in the present experiments was 5 μg/kg b.w./min, which is about equal to the maximal release rate from the cat's adrenal medulla (Celander 1954). For the cat it seems reasonable to conclude therefore that physiological doses of adrenaline can give substantial increases in pulmonary capillary pressure, without any pooling of blood in the lungs. Left ventricular failure has not been involved as there was no or only small increases in left atrial pressure.

Hypoxia was included in the present experiments because hypoxemia was regarded to be very potent stimulus for generalized sympathetic discharge and for adrenal catecholamine release. Hypoxemia and increased sympathetic activity appear to be essential events in many critically ill and injured patients suffering from the so-called wet lung syndrome. At the same time ventilation hypoxia will have a direct vasomotor effect on the lungs. Alveolar hypoxia will thus cause pulmonary constriction and hypertension through a specific mechanism involving histamin (Hauge and Staub 1969). Wayne and Severinghouse (1968) have shown that hypoxia-induced pulmonary vasoconstriction is located in precapillary vessels. The resulting effect on the pulmonary vasculature of ventilation hypoxia on the one hand and of an increased sympathetic discharge rate and of increased adrenal catecholamine release on the other cannot easily be predicted.

In the present experiments the P_{O_2} was kept as low as 25 mm Hg for half an hour but this very grave hypoxemia was well tolerated. The cardiac output was slightly increased throughout the experiment, and the femoral arterial pressure showed only a very moderate reduction (to about 100 mm Hg) at the end of the experiment. The left atrial pressure increased by 2–3 mm Hg. Left cardiac failure was thus apparently not evolving. The hypoxemia gave an increase in pulmonary arterial pressure by some 75% (Fig. 1 C). Both the blood volume and the extravascular water content of the lungs were, if anything, reduced after half an

found to be reduced by about 25% during hypoxemia ($p = 0.001$). The extravascular lung water content changed in the same direction as was the case during infusion of noradrenaline Thus the water content in the lung after 28 min with hypoxia seemed to be slightly reduced as compared to the situation in the control animals ($p = 0.055$) The left atrial pressure increased by a mean value of only 2–3 mm Hg during the hypoxic period.

Discussion

When catecholamines are given to the intact animals, the pulmonary vascular changes seen will reflect both direct effects on the lung vessels, as well as influences on these vessels secondary to effects in the systemic circulation or on the right heart. In isolated, perfused cat and rabbit lungs Hauge, Lunde and Waaler (1967) have demonstrated that both adrenaline and noradrenaline reduce (through an α-receptor mechanism) pulmonary vascular volume The two drugs differed in their effects on the resistance vessels, as adrenaline caused dilatation (β-receptors) and noradrenaline predominantly constriction (α-receptors). When catecholamines are given to the intact animal, results have been achieved which suggest that vasoconstriction of blood vessels in the systemic circulation can cause translocation of blood to the distensible low-pressure vessels of the lesser circulation (Luisada 1967)

In the present experiments, however the direct effects of the infused catecholamines on the pulmonary capacity vessels seemed to dominate over volume changes secondary to systemic effects of the drugs. Our findings are thus in agreement with those Hauge *et al* (1967) reported from isolated perfused lungs. In contrast to our findings Feelay *et al* (1963) observed in intact dogs that the systemic effects of catecholamines predominated with increased pulmonary blood volume as a consequence.

As in the present experiments pulmonary blood volume did not increase during catecholamine infusions, although the vascular distending pressures were elevated, active vasoconstriction must have taken place. It thus appears that the pulmonary vascular bed can resist distension with unchanged, or even reduced resistance

It has been debated whether recruitment of new vessels or distension of already open ones, are the important determinant for pulmonary blood volume as intravascular pressures change (Permutt *et al* 1969) A large fraction of the pulmonary vascular resistance is located in the arteries. Therefore, with reduced vascular resistance, as seen during infusion of adrenalin (Fig. 1 a), a pressure elevation must have prevailed throughout the arterial tree. Since there was no increase in pulmonary blood volume in this situation significant vascular recruitment can apparently not have taken place It follows that recruitment is unlikely to be an universal determinant for blood volume in cat lungs in situ, as it may be in dog lungs according to Permutt *et al* (1969)

When compared to the values from the control group the animals given adrenalin for 28 min had a 4% increase in extravascular lung water content, whilst the animals given noradrenalin for the same period showed a small decrease in this parameter The difference between the groups of infused animals in this respect was significant, $p = 0.026$. Changes in extravascular water content in the lungs might reflect changes in hydrostatic capillary pressure. This had been demonstrated in isolated perfused rabbit lungs (Aarseth and Waaler

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The Importance of Asphyxia for the Development of Diving Bradycardia in Ducks

By

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Abstract

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Upon submersion, the domestic duck normally displays prompt but modest cardiac retardation, which, after short delay is reinforced into pronounced state of bradycardia. The present study has revealed that arterial asphyxia is prerequisite for the reinforcement of the initial slight bradycardia.

Once diving animal has overcome the immediate danger of drowning upon water immersion by cessation of breathing, it has to encounter the thereby incurred risk of asphyxiation. It is known that the most prominent adjustment displayed by diving vertebrates during such underwater episodes is their unusual ability to redistribute their blood in a way which greatly delays the exposure of brain and myocardium to asphyxia (Irving 1938). In principle this implies transformation of their cardiovascular system into "heart-brain-lung preparation (Folkow 1968), delivering almost the total oxygen reserves in blood and lungs to these vitally important tissues. The integration of the various mechanisms initiating and reinforcing this neurogenic response (*i.e.* vasoconstriction and bradycardia) are, on the other hand, in several respect poorly understood. Hollenberg and Uvnäs (1963), Jones and Purves (1970), and Holm and Sørensen (1972) have investigated the consequences of carotid body denervation upon the cardiac responses to head immersion in the duck. They suggest on the basis of their results that the arterial chemoreceptors are mainly responsible for the cardiovascular responses to diving. It can be argued, however that the procedures used may not have included denervation of all chemosensitive receptors, and/or that the denervation has affected also other types of receptors (*e.g.* baroreceptors) that may be of great importance for the cardiovascular adjustments during diving. In order to get additional information about the importance of peripheral chemoreceptors for the establishment of the diving response, an experimental set up has been designed in which ducks can be exposed to head immersion and the associated apnoe without being exposed to asphyxia.

hour with this hypoxemia, indicating a strong precapillary vasoconstriction. The pres experiments therefore lend no support to the hypothesis that direct vascular changes cau by the combined actions of hypoxemia and catecholamines are important etiological fact for the development of the wet lung syndrome. As has previously been shown, increa levels of catecholamines do play an indirect role in this connection, through interactions the hormones with blood platelets (Bo and Hognestad 1971).

The conclusions from the present work are several In situations where an increase in t level of circulating adrenaline is a predominant feature, a moderate increase in lung wa content may result from the various circulatory adjustments to adrenaline, tending to gi an increase in pulmonary capillary pressure. In situations where α-receptors are marked stimulated, and in situations with increased sympathetic activity combined with hypoxemi precapillary vasoconstriction will however be overwhelming, thus preventing an increase the hydrostatic lung capillary pressure. Other factors than direct adrenergic effects on lu vessels must apparently be responsible for the accumulation of interstitial water in the lu tissue under such conditions. The key role played by blood platelets in the evolvement lung changes during hemorrhage shock (Bo and Hognestad 1971) should be remember here.

References

AARSETH, P Reduction in pulmonary blood volume after a blood loss. *Acta physiol. scand* 1970. 459–469

AARSETH P and G Bo, Content of blood and of extravascular water in cat lungs dur g changes i tot blood volume. *A ta physiol. scand.* 1972. *85* 343–352.

AARSETH, P and B A. WAALER Interstitial water content in isolated rabbit lungs at different levels capillary pressure. *Acta physiol scand.* 1974 Suppl. 396. 76.

AARSETH, P G Bo and A. H UGE. I preparation.

BEAN J W and D L. BECKMAN, Centrogenic pulmonary pathology in mechanical head i jury *J ap Physiol* 1969 *27* 807–812.

Bo, G a d J HOGNESTAD The role of the adrenal medulla n posthemorrhagic pulmonary hypertensio *Acta physiol. scand* 1971 *83* 124–132.

CELANDER, O The range of co trol exercised by the "sympathico-adrenal system *A ta ph siol sca* 1954 Suppl 116.

ERBOZ, N and S C. FINESTONE, Adrenaline induced pulmonary oedema and its treatment *Brit J Anaes* 1971 *43* 709–712.

FEELY J W T D LEE and W R. MILNOR, Active and passive components of pulmonary vascular respo to vasoactive drugs in the dog. *Amer J Physiol* 1963 *205* 1193–1199

H UGE, A. and N C. ST UB, Prevention of hypoxic vasoconstriction cat lung by histamine-releas agent 48/80, *J appl. Physiol.* 1969 *26* 693–699

HAUGE, A., P M K LUNDE and B. A. WAALER, Effects of catecholamines on pulmonary blood olum *Acta physiol scand.* 1967 *70* 323–333

KARLSEN J G Bo and P AARSETH, The volumes of blood nd of extravascular w ter in the lungs of ca during periods of acute hypoxemia. *Microvasc Res.* 1973 *6.* 256.

LUISADA, A. A., Mechanism of neurogenic pulmonary edema. *Amer J Cardiol* 1967 *20* 66–68

MOORE, F D J H LYONS, E. C. PIERCE, A P MORGAN P A DRINKER, J D MACA THU and G. DAMMIN *Post-traumatic pulmonary insufficiency* W B. Saunders C mpany Philadelphia 1969

PERMUTT S., P CALDINI, A. M SERI, W H P LMER T SASAMORI and K ZIE LE Recruitment ersu distensibility in the pulmonary vascula bed. I *The pulmonary circulation and interstitial space* Ed b A. P Fishma and H H Hecht. The U iversity of Chicago Press, Chicago 1969

WHAYNE, Th F and J W SEVERINGHAUS, Experimental hypo ic pulmonary edema the cat *J app Physiol* 1968 *25* 729–73..

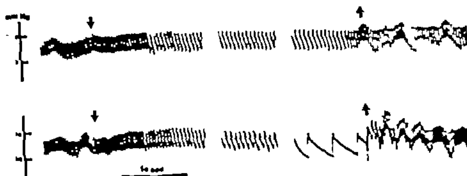


Fig. 1. The typical blood pressure response to 1 min head immersion in duck. The recordings were made the beginning, after 30 s, and at the end of the dive. *Upper tracing*: The response to non-asphyxic submersion (like the lower tracing of Fig. 2). *Lower tracing*: The response to submersion with asphyxia, the lungs being perfused by gas mixture of 3% O_2 in N_2.

Table I). Largely normal diving responses were also obtained when the lungs were perfused with O_2–N_2 mixture of low oxygen concentration (3%) provided the animal did not struggle (Fig. 3). The delay of the response in this experiment might be due to the fact that some oxygen was supplied. Perfusion with pure nitrogen led to such vigorous struggling that the diving response was partly masked.

If the lungs were instead "ventilated" by a retrograde influx of 20 % oxygen in nitrogen to the air sac during submersion, resulting in a maintenance of normal or slightly super normal arterial PO_2 (usually associated with slight hypocapnia, as revealed by an arterial pH of 7.49 ± 0.05 (S.E., n 9), they all responded to head immersion with the usual apnoe and a quiet behaviour. The cardiovascular response to submersion, was, however now strikingly different from that of a normal dive. As seen from Fig. 2, the heart rate fell almost instantaneously upon submersion to about 60% of the pre-dive value, but did not further decrease as long as the oxygen delivery *via* the air sac continued, no matter how long the dive continued. Conversely upon emersion the heart rate promptly returned to the pre-dive level without the usual overshoot in terms of a transient pressure rise and tachycardia (Table I)

TABLE I. Heart rate of ducks, before, after 60 s, and after 5 of recovery from normal and non-asphyxic dives, all lasting for 90 s. The numbers represent means and standard error of the mean (S.E.). Included are also the calculated per cent of pre-dive value (%), for each group.

	Before dive M ± S.E.	After 60 dive		After 5 recovery	
		M ± S.E.	% ± S.E.	M ± S.E.	% ± S.E.
Normal	251 ± 40 (5)	24 ± 4 (5)	9 ± 2 (5)	327 ± 29 (5)	140 ± 23 (5)
Nonasphyxic	233 ± 30 (9)	132 ± 14 (9)	59 ± 3 (9)	247 ± 16 (9)	113 ± 5 (9)



Fig. 1 Arterial blood pressure of a duck breathing air at surface position. Unidirectional, retrograde and nonpulsatile left lung ventilation with air (inlet pressure 1.5 cm H_2O), performed between arrows.

Material and methods

Eight domestic (Pekin) ducks of both sexes, weighing 2 3 kg, none of which displayed spontaneous breathing through their tracheostomy while submerged, were used in this study The trachea was cannulated with a 4.5 mm Flotex® endotracheal tube, and an intravascular catheter (PP 100) was inserted into the brachial artery Another polyethylene catheter (inner diameter = 3 mm) with side holes was advanced through the thoracic wall into the right posterior thoracic air sac, care beeing taken to achieve an air tight seal around the cannula and to avoid bleeding (post mortem inspection). All cannulations were performed under local (Xylocain® 1 %) anesthesia.

During the experiment the animal was restrained to a board ventral side down. The arterial catheter was used for pressure recording (Statham transducer P23H) and blood sampling and arterial PO_2 and pH were analysed by use of specific Radiometer electrodes. The air sac catheter was connected to a modified spirometer whereby the animal could be oxygenated by sending a stream of oxygen (?0 %) in nitrogen in a retrograde direction through the lung, at a nonpulsatile inlet pressure of 1.5 cm H_2O Diving was simulated by gently tilting the board so that the head was submerged in a water pool the water temperature is kept at 25°C. In order to secure unidirectional gas flow through the system during the dive, a one way valve was connected to the end of the tracheal tube at the very moment of submersion. After recording the normal cardiovascular responses to head immersion *i.e.* without oxygenation ("diving with asphyxia"), the procedures were repeated while the lungs were kept oxygenated through the air sac cannula ("diving without asphyxia").

Results

During normal breathing in the pre-dive situation, onset of the unidirectional, retrograde and non-pulsatile pulmonary "ventilation" did not *per se* affect significantly either blood pressure, heart rate or respiratory rate (Fig. 1). Diving, without this retrograde pulmonary ventilation resulted in typical respiratory and cardiovascular responses to submersion with an initial modest bradycardia that was greatly reinforced during prolonged immersion (Fig. 2,

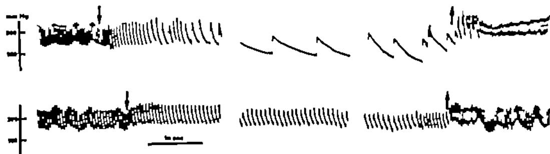


Fig. 2. The typical blood pressure response to 2 m head immersion in a duck. The recordings were made at the beginning, after 60 s, and at the end of the dive. *Upper tracing* The normal response (without any kind of ventilation). *Lower tracing* The response of the same animal, while being ventilated as described for Fig. 1 (with oxygen/nitrogen mixture: a non-asphyxic dive).

Hollenberg, N. K. and B. Uvnäs, The role of the cardiovascular response in the resistance to asphyxia of avian divers. *Acta physiol. scand.* 1963. *58*, 150–161.

Holm, B. and S. C. Sørensen, The role of the carotid body in the diving reflex in the duck. *Resp. Physiol.* 1972. *15*, 302–309.

Irving, L., Changes in the blood flow through the brain and muscles during the arrest of breathing. *Amer. J. Physiol.* 1938. *122*, 207–214.

Jones, D. R. and M. J. Purves, The carotid body in the duck and the consequences of its denervation upon the cardiac responses to immersion. *J. Physiol.* (Lond.) 1970. *211*, 279–294.

Leitner, L.-M. and M. Roumy, Vagal afferent activities related to the respiratory cycle in the duck: sensitivity to mechanical, chemical and electrical stimuli. *Resp. Physiol.* 1974. *22*, 41–56.

Öberg, B. and P. Thorén, Studies on left ventricular receptors, signalling in non-medullated afferents. *Acta physiol. scand.* 1972. *85*, 145–163.

Discussion

The essential finding of the present investigation is that ducks display a prompt, but quite modest bradycardia response when exposed to submersion provided arterial hypoxia is avoided. This suggests that the initial bradycardia is independent of asphyxia. Bradycardia also occurs, although to a lesser extent, even when ducks are artificially ventilated with air at normal tidal volumes during diving (Feigl and Folkow 1963) However it has been shown in geese (Cohn *et al* 1968) and we have found it to be true of some ducks also that both the initial and the delayed bradycardia component are closely associated with apnoe. It seems likely therefore, that the first event in diving is an afferent input from peripheral receptors (Andersen 1963) which induces an apnoeic state. This, in turn, causes a prompt but limited bradycardia, which is slightly enhanced due to the absence of an afferent input from lung receptors, like those described *e.g* by Leitner and Roumy (1974)

It has been shown previously (Blix and Berg 1974), that the delayed intensification of the initial diving bradycardia can be elicited by pure arterial hypoxia (even combined with a slight hypocapnia) This could indicate that the pronounced bradycardia arises as a primary chemoreceptor response (Daly and Scott 1963). However Blix *et al.* (1974) and Andersen and Blix (1974) have demonstrated that the delayed intensification of the bradycardia does not occur if the peripheral neurogenic vasoconstriction is abolished These findings may point to an important involvement also of high-and low pressure mechanoreceptors in the production of the intensified diving bradycardia in ducks. In respect of this, the left ventricular "volume receptors" described by Öberg and Thorén (1972) may be of great relevance.

In conclusion, the delayed pronounced bradycardia, unlike the initial cardiac retardation of diving ducks, seems to be closely associated with arterial asphyxia. The manifestation of the response is, however in some manner dependent upon the chemoreceptor initiated peripheral vasoconstriction

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I wish to thank Mr K. A. Stokkan and Miss B Larsen for their ample assistance during the experiments.

References

ANDERSEN, H T The reflex nature of the physiological adjustments to diving, and their afferent pathway *Act physiol scand.* 1963 *58* 263–273

ANDERSEN H T and A. S. BLIX, Pharmacological exposure of components in the autonomic control of the diving reflex. *Acta physiol scand* 1974 *90* 381 386.

BLIX, A S and T BERG, Arterial hypoxia and the diving responses of ducks. *Acta physiol. scand.* 1974 *92*, 566–568.

BLIX, A S., E. L. GAUTVIK and H. REFSUM, Aspects of the relative roles of peripheral vasoconstriction and vagal bradycardia in the establishment of the diving reflex in ducks. *Acta physiol scand.* 1974 *90* 289–296

COHN J E., J KROG and R. SHANNON, Cardiopulmonary responses to head immersion in domestic geese. *J appl Physiol.* 1968, *25* 36–41

DALY M de BURGH and M J SCOTT The cardiovascular responses to stimulation of the carotid body chemoreceptors in the dog. *J Physiol.* (Lond.) 1963 *165* 179–197

FEIGL, E. and B. FOLKOW Cardiovascular responses in diving and during brain stimulation in ducks. *Acta physiol. scand.* 1963. *57* 99–110.

FOLKOW B., Circulatory adaptations t diving in ducks. *Proc Intern. Union. Ph iol Sci.* 1968 6 23–4

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The purpose of the present investigation was to study the extent and degree of specificity of the acute removal of circulating tritium-labelled NA from the blood stream, during one circulation through the lungs, and through a well-defined systemic vascular bed (forearm musculature), in man. A mixture of ^3H-( )-NA, ^{14}C-inulin and ^{125}I-albumin was injected proximally to the vascular bed under study and serial blood samples were rapidly drawn from the effluent distally to it. The disappearance of ^3H relatively to ^{125}I was taken as a measure of total (unspecific + specific) removal of ^3H NA, that of ^{14}C relatively to ^{125}I as a measure of filtration diffusion and that of ^3H relatively to ^{14}C as a measure of specific removal of ^3H-NA.

Material and Methods

Infusion of markers sampling of blood

18 healthy male subjects, 20–35 years old, whose informed consent was obtained, were used for the experiments. T block thyroid uptake of the ^{125}I used in the experiments, 50 mg NaI was given orally the day before.

In the study of removal of circulating NA during single passage through the pulmonary vascular bed (10 expts.) the bolus injection of the mixture of ^3H NA, ^{14}C-inulin and ^{125}I-albumin (*vide infra*) was made through catheter introduced percutaneously through the right antecubital vein and under fluoroscopic control guided into the right atrium near the tricuspid valve. For sampling blood after one circulation through the lungs second percutaneous catheter was introduced into the right brachial artery.

In the study of systemic vascular bed (8 expts.) the injection was made through catheter in the brachial artery and blood was sampled from catheter introduced in the distal direction into a deep vein of the forearm. A cuff was applied around the wrist and inflated to pressure of 220 mm Hg to exclude hand circulation. With this arrangement blood sampled from the deep vein mainly represents effluent from muscle tissue (Coles *et al.* 1958).

Immediately before injection, the three radioactive compounds, 10 μCi ^3H-( )-NA (New England Nuclear sp. 6.75 Ci/mmol), 5 μCi ^{14}C-labelled inulin (*New England Nuclear* inulin-carboxyl-^{14}C, sp. a. 1.3 mCi/g) and 20–30 μCi ^{125}I-labelled human serum albumin (human serum albumin, Kabi, labelled with ^{125}I, New England Nuclear the extent of labelling approximately one atom of iodine per protein molecule, and the $^{125}I/^{127}I$ ratio was about 10^{-4}; the labelled albumin was separated from contaminating unbound isotope by gel filtration on Sephadex), were mixed and diluted to 5 ml saline. After removing 5 ml of control blood 4.5 ml volume of the mixture was rapidly injected into the right atrium, or into the brachial artery respectively. A series of six 5 ml samples of blood were then drawn, at the respective sampling point, with about 15 intervals between successive samples, starting 5 or 15 after the injection, in the experiments with the systemic or the pulmonary vascular bed, respectively.

Analytical

All blood samples were immediately chilled on ice, after mixing with 1 ml 0.9 NaCl containing 0.1 EDTA to prevent clotting and 10 μg unlabelled NA to protect the labelled NA in the samples.

T increase the accuracy of the estimation of the degree of metabolic degradation of the injected ^3H NA during the single passage through the two vascular beds tested, 0.5 ml volume of the injection mixture remaining in the syringe was diluted ten-fold with saline containing 0.1 EDTA and 10 μg unlabelled NA per ml, and 0.5 ml of this solution was added to each of the two control blood samples drawn immediately before the injection. These controls were carried through all the various preparative and analytical steps together with the five 'test' samples.

After centrifugation for ten min in chilled laboratory centrifuge (Christ, swing-out buckets, 3 000 g_{max}) to remove blood cells, 2–3 ml volume of plasma was pipetted off. One ml of this was used for determination of total ^{125}I activity. T one ml of the remainder was added 0.1 ml 4 M perchloric acid to precipitate protein, including ^{125}I-labelled albumin. After centrifugation the protein-free supernatant was used for determination

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Specific and Unspecific Removal of Circulating Noradrenaline in Pulmonary and Systemic Vascular Beds in Man

By

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Abstract

STJÄRNE, L., L. KAISER, A. MATIĆ and G. BIRKE. *Specific and unspecific removal of circulating noradrenaline in pulmonary and systemic vascular beds in man.* Acta physiol. scand. 1975. 95. 46–53.

In order to study the extent and degree of specificity of the removal of circulating noradrenaline (NA) from the blood stream, during a single passage through the vascular beds of the lungs and of the forearm musculature of unanaesthetized healthy young men, the following radiometric method was used. A mixture of ^{3}H NA with ^{14}C inulin and ^{131}I labelled human serum albumin was injected proximally to the bed under study; six serial blood samples were drawn distally to it, with about 1.5 s interval, and the disappearance of ^{3}H NA relatively to that of ^{14}C inulin and ^{131}I albumin was determined. ^{3}H NA was found to be removed from the blood stream in both these vascular beds. However, in the forearm the removal of ^{3}H NA was much greater, and apparently to a large extent due to specific trapping in the tissues, since the clearance of ^{3}H NA over the vascular bed of the forearm markedly exceeded that of ^{14}C inulin. During passage through the lungs removal of ^{3}H NA was less marked, and apparently largely unspecific, since it was initially essentially equal to that of ^{14}C inulin. This suggests that removal of circulating ergones of small molecular size, from the blood stream passing through the lungs, may occur initially by unspecific filtration/diffusion. Their subsequent fate would depend on whether the extraluminal tissues, supplied by the lung circulation, possess specific mechanisms for concentrating and/or inactivating each particular compound.

As early as 1905 Elliott concluded that "adrenalin disappears in the tissues which it excites". Later experiments have confirmed that circulating adrenaline (A) (Burn 1932) and noradrenaline (NA) (Celander and Melander 1955) are extensively removed from the circulation during single passages through various vascular beds: both A and NA have been shown to have a half-life in the circulation of less than 20 s, or less than one circulation time (Ferreira and Vane 1967). While several systemic vascular beds have been found to be much more efficient than the pulmonary vascular bed in removing circulating catecholamines (Elliott 1905, Celander and Melander 1955, Ferreira and Vane 1967), there are reports in

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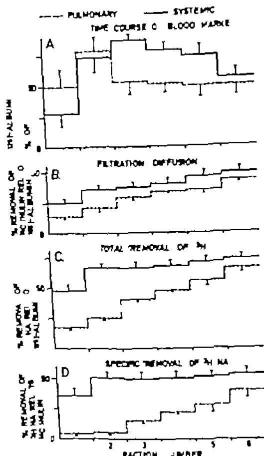


Fig 1 A Time course of appearance of ^{131}I-labelled albumin, used as marker for (non-diffusible compounds in) "blood" at the respective sampling points (for pulmonary circulation brachial artery for systemic circulation deep vein of the same arm, into the brachial artery of which the injection was made). Means ± S E (in panels A–D) B. Per cent removal of ^{14}C-inulin in relation to ^{131}I-albumin. If the ^{14}C/^{131}I ratio in the test sample equals that of the control, that would imply 0 removal of ^{14}C-inulin. If ^{14}C in the test sample falls to 0, removal of ^{14}C-inulin would be 100 %. Removal of ^{14}C-inulin relatively to ^{131}I-albumin is regarded as measure of elimination of solutes of small molecular size, by filtration + diffusion C Per cent removal of ^{3}H-NA, in relation to ^{131}I-albumin (same type of calculation as above), regarded as measure, in the first 2–3 fractions, of total removal of ^{3}H NA from the blood stream, during one passage through the respective vascular bed. D Per cent removal of ^{3}H-NA, in relation to ^{14}C-inulin (same type of calculation as in B) If the ^{3}H-NA/^{14}C-inulin ratio in the test sample equals that of the control (in which case the change in ^{3}H NA/^{131}I-albumin ratio, after passage through the respective vascular bed, would equal that in ^{14}C-inulin/^{131}I-albumin ratio), total removal of ^{3}H-NA would equal total removal of ^{14}C-inulin. Since ^{14}C-inulin-removal is assumed to occur by unspecific mechanisms, ^{3}H-NA removal would in that case also be entirely unspecific (specific removal of ^{3}H-NA would be 0 %) If the ^{3}H-NA/^{14}C-inulin ratio is reduced to e.g. one half, this would imply that ^{3}H NA is removed twice as efficiently as ^{14}C-inulin, of the total amount of ^{3}H-NA eliminated from the blood stream 50 % would be removed 'unspecifically' (by filtration/diffusion), and 50 % 'specifically' by additional specific trapping in the tissues.

$$\left[1-\frac{\left[\frac{^{14}C}{^{131}I}\right]\text{Test}}{\left[\frac{^{14}C}{^{131}I}\right]\text{Control}}\right]100$$

of total ³H and ¹⁴C, and for cation exchange column chromatography (Amberlite CG 120, Type 2, 200–400 mesh, columns 6 cm in height and 0.5 cm in diameter, eluant 1 M hydrochloric acid), for separation of labelled NA from its various metabolites (*cf.* Stjärne 1966).

To determine ³H and ¹⁴C 0.4 ml aliquots of the different fractions were counted in 10 ml of a 7:3 mixture of toluene with ethanol, containing 4 g of PPO and 0.1 g of POPOP per liter toluene. ³H and ¹⁴C in the mixture were determined by simultaneous dual channel counting for 10 min, with correction for "spillover" in an ABAC SL40 Intertechnique Liquid Scintillation Spectrometer. ¹³¹I activity was determined with a scintillation detector, the pulse height analyzer of which was adjusted to a maximum sensitivity at 0.36 MeV.

Premises

The aim of the experiments was to study the acute removal of ³H NA, during one passage through different vascular beds. ³H NA was injected together with ¹³¹I labelled albumin, used as a marker for substances not leaving the blood stream, and ¹⁴C labelled inulin, as a marker for substances "freely" diffusible and filterable, through the capillary walls.

The fall in ³H/¹³¹I ratio, in blood sampled distally to the vascular bed under study, when compared to that in control blood samples to which the isotope mixture was added *in vitro*, should thus, independently of the absolute levels of ³H or ¹³¹I, reflect total removal of ³H NA from the blood stream. The fall in ¹⁴C/¹³¹I ratio should reflect unspecific removal of diffusible solutes from the blood stream. And, provided that ³H NA and ¹⁴C inulin disappear from the blood stream to an equal extent in tissues lacking specific trapping mechanisms for ³H NA, a reduction in the original ³H/¹⁴C ratio should give a measure of the specific removal of ³H NA.

Results

Time course of appearance of ¹³¹I at sampling points

The results in this respect are expressed in relative terms. The peak level of ¹³¹I in each experiment was given the value of unity and the ¹³¹I activity in the other sampling periods of that experiment were normalized with respect to this. As shown in Fig. 1A, the peak was most frequently reached in the second sampling period in the pulmonary circulation and in the third period in the systemic bed under study.

Since these peak periods reflect mean transit time, it follows that mainly data obtained from them are of relevance to the problem under study, since they represent removal during a single passage through the respective vascular bed.

Changes in the ³H/¹³¹I ratio

During a single passage through the forearm vascular bed the ³H/¹³¹I ratio was immediately strongly reduced. In the first few samples taken after one circulation through the pulmonary bed it fell only moderately (the difference from the corresponding values in the systemic bed during each of the first four sampling periods was highly significant, $p < 0.001$, $n = 13$–18), when compared to *in vitro* controls, but in later samples it progressively declined to approach the level observed in the samples from the systemic bed. Thus the total removal of ³H NA was 65–70% (from the second period and onwards) during one passage through the bed of the forearm, while total removal of ³H NA in the pulmonary circulation was initially much lower, progressively increasing from 17.5% in the first sample to 65% in the last (Fig. 1C, where the results are plotted as the deficit in ³H relatively to ¹³¹I or per cent total removal of ³H NA).

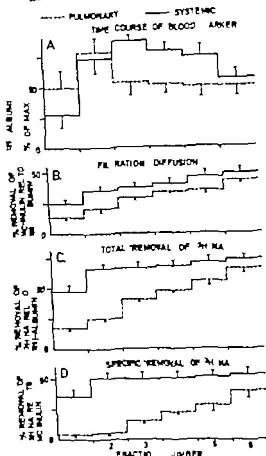


Fig. 1. A. Time course of appearance of ^{131}I-labelled albumin, used as a marker for (non-diffusible components in) "blood" at the respective sampling points (for pulmonary circulation, brachial artery; for systemic circulation, deep vein of the same arm, into the brachial artery of which the injection was made). Means ± S.E. (in panels A–D). B. Per cent removal of ^{14}C-inulin in relation to ^{131}I-albumin. If the ^{14}C/^{131}I ratio in the test sample equals that of the control, that would imply 0% removal of ^{14}C inulin. If ^{14}C in the test sample falls to 0, removal of ^{14}C-inulin would be 100%. Removal of ^{14}C-inulin relatively to ^{131}I-albumin is regarded as a measure of elimination of solutes of small molecular size, by filtration + diffusion. C. Per cent removal of ^{3}H NA, in relation to ^{131}I-albumin (same type of calculation as above), regarded as a measure, in the first 2–3 fractions, of total removal of ^{3}H NA from the blood stream, during the passage through the respective vascular bed. D. Per cent removal of ^{3}H-NA, in relation to ^{14}C-inulin (same type of calculation as in B). If the ^{3}H-NA/^{14}C-inulin ratio in the test sample equals that of the control (in which case the change in ^{3}H-NA/^{131}I-albumin ratio, after passage through the respective vascular bed, would equal that in ^{14}C-inulin/^{131}I-albumin ratio), total removal of ^{3}H-NA would equal total removal of ^{14}C-inulin. Since ^{14}C-inulin-removal is assumed to occur by unspecific mechanisms, ^{3}H-NA removal would in that case also be entirely unspecific (specific removal of ^{3}H NA would be 0%). If the ^{3}H-NA/^{14}C-inulin ratio is reduced to e.g. one half, that would imply that ^{3}H-NA is removed twice as efficiently as ^{14}C-inulin, of the total amount of ^{3}H NA eliminated from the blood stream 50% would be removed 'unspecifically' (by filtration/diffusion), and 50% 'specifically' by additional specific trapping in the tissues.

$$\left[1 \frac{\left[\frac{^{14}C}{^{131}I}\right] \text{Test}}{\left[\frac{^{14}C}{^{131}I}\right] \text{Control}} \right] 100$$

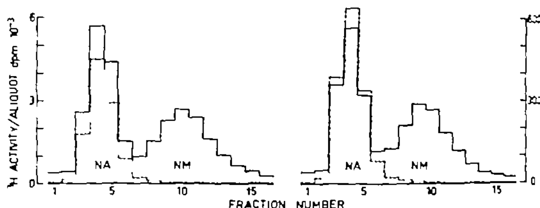


Fig. 2. Typical examples of ion exchange chromatograms of perchloric acid extracts of plasma from cont and test samples. For further details see the Text.

Changes in C/¹²⁵I ratio

A progressive fall in the ^{14}C/^{125}I ratio reflecting the degree of non-specific 'removal' diffusible solutes from the blood stream, was observed in both the pulmonary and the sys mic bed (Fig. 1 B). Although the removal of ^{14}C inulin during a single passage through t lungs was smaller than that during one circulation through the forearm ($p < 0.001$ pool data, $n = 103$) the results still indicate that diffusible solutes to a considerable extent do lea the circulating blood stream in the vascular bed of the lungs as well as in that of the forear

Changes in H/¹⁴C ratio

The changes in ^{3}H/^{14}C ratio in the two vascular beds were very markedly different ($p < 0.0$ for the first three sampling periods, $n = 13$–18 in each). After one passage through the for arm the fall in this ratio was about 50 %, whereas passage through the lungs did initially the first two samples, not change the ^{3}H/^{14}C ratio at all, when compared to *in vitro* contro In later samples it progressively fell to approach the levels seen in the systemic bed (c Fig. 1 D).

Identity of ³H in blood samples

Ion exchange chromatographic analysis of perchloric acid extracts of plasma from bloo taken from the sampling points gave exactly the same results as that of control blood sampl to which the radioactive mixture was added *in vitro* (*cf* under Methods). In all cases a sing peak of ^{3}H activity was obtained, exactly coinciding with the fluorescence peak of adde carrier NA (Fig. 2). The effluent from the columns contained $0.98 \pm 0.57\%$ ($n = 4$) an $1.75 \pm 0.91\%$ ($n = 8$) of the added ^{3}H activity in controls and test samples, respectively. N ^{3}H activity peak was observed coinciding with normetanephrine. The total recovery of ^{3}

y in the ³H NA peak in the test samples was identical with that in the controls. It may re be concluded that all of the ³H appearing at the respective sampling points, during rt time of observation, represented unchanged ³H NA.

Discussion

aim of the present study was to compare the acute removal of ³H NA from the blood , during one circulation, through the pulmonary and one systemic vascular bed, in

results clearly show that, in man, ³H-NA is removed from the blood stream in both ar beds under study although much more promptly and efficiently from that of the m than from that of the lungs. That removal of catecholamines is more marked in ic vascular beds has previously been observed in various experimental animals tt 1905, Celander and Melander 1955 Ferreira and Vane 1967). That some catechol- removal occurs in the lesser circulation as well has also been previously observed, perimental animals (Elliott 1905, Eisenmann *et al.* 1964, Hughes *et al.* 1969 Ginn and 1972) as well as in man (Biron *et al.* 1969 Gillis *et al.* 1972).

the forearm the removal of ³H-NA was nearly twice as great as that of ¹⁴C-inulin, sting the presence of specific trapping mechanisms for ³H-NA in these tissues how- the present results give no indication as to the identity or cellular localization of such anisms (*cf* Iversen 1971 Ginn and Vane 1969 Gryglewski and Vane 1970, Osswald Branco 1973).

the lesser circulation the removal of ³H NA was far less prompt and efficient than that e systemic bed, and about equal to that of ¹⁴C-inulin, in the first two sampling periods. suggests that ³H NA was initially removed from the pulmonary blood stream by un- ific diffusion/filtration.

he present results give no clue as to the ultimate fate of the NA removed from the pul- ary circulation, in man. But results obtained in studies of isolated perfused rat or rabbit s indicate that the NA removed from the pulmonary vascular bed in these species is vely taken up into sites in which it is metabolized rather than stored, histofluorescence dies appear to implicate capillary endothelial cells as the probable cellular sites of uptake ghes *et al* 1969 Alabaster and Bakhle 1973 Iwasawa *et al.* 1973 Iwasawa and Gillis 4).

That considerable transcapillary exchange of water does occur even in the pulmonary ulation has been demonstrated in experiments with isolated perfused lungs from various cies (Guyton and Lindsey 1959 Hauge *et al.* 1966) as well as *in vivo* in anaesthetized dogs inard and Enns 1954) or unanaesthetized sheep (Staub 1971). I the present study in un- esthetized man, a considerable and statistically highly significant fall in the ¹⁴C-inulin/ I-albumin ratio was observed after one passage through the lungs, although less marked n after one passage through the vascular bed of the forearm. This finding is apparently variance with the observation of Chinard and Enns (1954) in anesthetized dogs, that the ne-concentration relationship for inulin was identical with that of the dye T 1824 used a marker for plasma protein, after simultaneous injection of the two compounds into the

jugular vein, and sampling after passage through the lungs. It is at present not possible to decide on the reason for the discrepancy which may be due to species differences, to anesthesia or to methodology However that may be, the present findings strongly suggest that considerable transcapillary exchange of diffusible/filterable solutes does occur in the pulmonary capillaries of unanesthetized man. This may well be the immediate mechanism of removal from the lung capillary lumen not only of NA, but also of other circulating ergones of small molecular size. The ultimate fate of the particular ergone would depend on whether or not the extra-luminal tissues, including the capillary endothelium, contain specific mechanisms for concentrating and/or inactivating each of these compounds.

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References

ALABASTER, V A and Y S. BAKHLE, The removal of noradrenaline in the pulmonary circulation of rat isolated lung. *Brit J Pharmacol.* 1973 47 325–331

BIRON, P J C BOILEAU and L. CAMPEAU, Norepinephrine inactivation in the pulmonary circulation of animals and humans (abstr). *Clin Res* 1969 17 230.

BURN, J H The action of tyramine and ephedrine. *J Pharmacol. exp Ther* 1932. 46 75–95.

CELANDER, O and S. MELLANDER, Elimination of adrenaline and noradrenaline from the circulating blood. *Nature* (Lond.) 1955. 176 973–974

CHINARD, F P and T ENNS, Transcapillary pulmonary exchange of water in the dog. *Amer J Physiol.* 1954 178 197–202.

COLES, D R., K. E. COOPER, R. F MOTTRAM and J V OCCLESHAW The source of blood samples withdrawn from deep forearm veins via catheters passed upstream from the median cubital vein *J Physiol.* (Lond) 1958. 142 323–328

EISEMAN, B., L. BRYANT and T WALTUCH, Metabolism of vasomotor agents by the isolated perfused lung. *J thorac cardiovasc. Surg* 1964 48 798–806.

ELLIOTT T R., The action of adrenalin. *J Physiol (Lond.).* 1905 32 401–467

FERREIRA, S. H and J R. VANE, Half lifes of peptides and amines in the circulation. *Nature (Lond.)*. 1967 215 1237–1240.

GILLIS, C. N N M. GREENE, L. H CRONAU and G L. HAMMOND Pulmonary extraction of 5-hydroxytryptamine and norepinephrine before and after cardiopulmonary bypass in man. *Circulat R* 1972. 30 666–674

GINN R. and J R. VANE, Disappearance of catecholamines from the circulation. (*Nature (Lond.)*. 1968 219 740

GRYGLEWSKI, R. and J R. VANE, The inactivation of noradrenaline and isoprenaline in dogs. *Brit J Pharmacol.* 1970. 39 573–584

GUYTON, A. C and A. W LINDSAY Effect of elevated left atrial pressure and decreased plasma protein concentration on the development of pulmonary edema *Circulat Res.* 1959 7 649–657

HAUGE, A., P K. M LUNDE and B. A. WAALER, Transvascular fluid balance the lung *J Physiol. (Lond)* 1966. 186. 94–95P

HUGHES, J C. N GILLIS and F E. BLOOM, Uptake and disposition of norepinephrine in perfused rat lung. *J Pharmacol. exp. Ther* 1969 169 237–48.

IWASAWA, Y and C. N GILLIS, Pharmacological analysis of norepinephrine and 5-hydroxytryptamine removal from the pulmonary circulation Differentiation of uptake sites for each amine *J Pharmacol. exp Ther* 1974 188 386–393

IWASAWA, Y C. N GILLIS and G AGHAJANIAN, Hypothermic inhibition of 5-hydroxytryptamine and norepinephrine uptake by lung: Cellular location of amines after uptake. *J pharmacol exp Ther* 1973 186. 498–507

...VERSEN, L. L., Role of transmitter uptake mechanisms in synaptic neurotransmission. *Brit. J. Pharmacol.* 1971 *41* 571–591

...HATHI, A. A., B. R. LEVINE and M. J. ANTONUCCI, Uptake of catecholamines in guinea-pig lung: Influence of cortisol and anaphylaxis. *J. Allergy clin. Imm.* 1975. *55* 170–181

...OSWALD, W. and D. BRANCO, The effects of drugs and denervation on removal and accumulation of noradrenaline in the perfused hindlimb of the dog. *Naunyn-Schmiedeberg Arch. Pharmacol.* 1973. *277* 175–190.

...STAUB, N. C., Steady state pulmonary transvascular water filtration in unanaesthetized sheep. *Suppl. to Circulat. Res.* 1971 *28–29*, 135–139

...STJÄRNE, L. Studies of noradrenaline biosynthesis in nerve tissue. *Acta physiol. scand.* 1966. *67* 441–454.

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Rat Liver Mitochondrial Enzyme Activities in Hypoxia

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Abstract

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R t liver mitochondrial enzyme activities were measured after exposing the animals to the atmospheric pressure of 380 mm Hg for 5 h and 14 days. Succinate dehydrogenase and succinate oxidase activities increased significantly during the hypoxic period of 14 days. No change was observed in cytochrome oxidase activity Malate dehydrogenase and glutamate dehydrogenase activities increased somewhat, but not significantly The efficiency of oxidative phosphorylation (the ADP O ratio) in the isolated mitochondria remained unchanged. *The exact mitochondrial protein values showed a 15% decrease* as compared with the control group The concentrations of cytochromes did not change significantly in the hypoxic group During the short hypoxic period succinate dehydrogenase, succinate oxidase and cytochrome oxidase activities increased as compared with those i the control group.

Key words. liver hypoxia, mitochondria, cytochromes, oxidative phosphorylation, succinate dehydrogenase

Mitochondria are resistant to low oxygen tension at the tissue level (Chance 1965). They can be synthesized in yeast in anaerobic conditions though these mitochondria are not functionally normal (Criddle and Schatz 1969) Low oxygen tension in the inspired air, however may result in damage in mitochondria, as can be observed in electron microscope studies (Glinsmann and Ericsson 1966) or radioisotope experiments (Rabinowitz *et al* 1971)

As a result of adaptation to low oxygen tension the oxygen-carrying capacity of the blood increases and the oxygen level in the various tissues remains relatively high. During moderate hypoxia mitochondria are capable of exhibiting normal functional metabolism (Park *et al* 1973) and it seems probable that no damage is done to them (Sanders *et al* 1965). It is evident that mitochondrial metabolism can adapt to these circumstances. The type and significance of this adaptation has not been resolved In addition there is much controversy over mitochondrial function in moderate hypoxia and during the adaptation process. In the present study mitochondrial enzyme activities have been investigated in 2 hypoxic periods of different length. Part of this investigation has been communicated in a preliminary form (Kinnula 1974).

Material and Methods

...star rats of both sexes (age about 4 months, weight 250–330 g) were used as experimental animals. ...poxia was produced with the aid of a hypobaric pressure chamber. Care was taken that in the same experiment the same sex and age ratios were achieved in both the control and hypoxic groups. The hypoxic group was exposed to an atmospheric pressure of 380 mm Hg corresponding to an altitude of about 5 500 ... Low pressure was obtained in the chamber in about 10 min. Rats were maintained in the chamber for ... in the acute experiments and for 14 days in the chronic experiments. The chamber was opened every ... days for a few minutes to weigh, feed and water the animals. Rats were given food (commercial standard rat pellets) and water *ad lib.* throughout the experiments.

After the hypoxic periods the rats were decapitated and blood samples were taken for hemoglobin and hematocrit determinations. The livers were rapidly removed and placed in 0.32 M sucrose-2 mM Tris solution (pH 7.4) at 0°C. The livers were homogenized with a glass homogenizer fitted with a motor-driven Teflon pestle. A sample from the total homogenate was taken for enzyme and cytochrome aa_3 determinations. The mitochondrial fraction was isolated according to O'Brien and Kalf (1967). One washing of the fraction was carried out at 5 200 g for 10 min. The mitochondrial pellet was used immediately.

Mitochondrial phosphorylation (ADP: O ratio) was determined polarographically by means of a Clark type oxygen electrode using succinate as substrate by the modified method of Hansson (1967).

Succinate dehydrogenase (EC 1.3.99.1.) was determined spectrophotometrically by 2 methods from the total homogenate and from the isolated mitochondria. In the first method potassium ferricyanide was used as an artificial electron acceptor and the determination was performed by the method of Bonner (1955). The dye concentration was checked in the laboratory so as to obtain the fastest reaction velocity possible. The specific activity is expressed as nmole/min/mg protein as calculated from the absorbancy change at 400 nm. In the second method dichloroindophenol was used as an artificial electron acceptor after Green *et al.* (1955). The specific activity is expressed as nmol/min/mg protein.

Succinate oxidase and cytochrome oxidase (EC 1.9.3.1.) activities were determined manometrically (Umbreit 1964). The oxygen uptake at 37°C was measured for 60 min after the initial equilibration of 5 min. The enzyme activities are expressed as μl O_2/min/mg protein.

Malate dehydrogenase (EC 1.1.1.37.) and glutamate dehydrogenase (EC 1.4.1.3.) activities were determined by measuring the rate of NAD⁺ reduction by the methods of Siegel and Englard (1961) and Beaufay *et al.* (1959). The activities are expressed as μmol NAD⁺ reduced/min/mg protein.

The cytochrome assays were performed with a double beam spectrophotometer (Perkin Elmer Double Beam 124). The reduction of cytochromes was accomplished in the presence of cyanide and antimycin A by the method of Chance and Williams (1955), as modified by Hallman *et al.* (1972). The calculations were performed according to Vanneste (1966). The cytochrome concentrations are expressed as nmoles/mg protein.

The exact mitochondrial protein values were calculated from the cytochrome aa_3 concentrations in the total homogenate and in the isolated mitochondria. Since cytochrome aa_3 is a specific mitochondrial component, assays of this cytochrome can be used to evaluate the amount of mitochondria in tissues (Schollmeyer and Klingenberg 1962).

Glucose-6-phosphatase (EC 3.1.3.9.), the marker enzyme of the microsomal fraction, was determined according to Leighton *et al.* (1968) with a slight modification. The inorganic phosphate released during the incubation period (30 min, 37°C) was determined according to Ernster *et al.* (1950).

Acid phosphatase (EC 3.1.3.2.), the marker enzyme of the lysosomal fraction, was determined by the modified method of Appelmans *et al.* (1955). The inorganic phosphate was determined as mentioned above. The activity is expressed as the amount of inorganic phosphate released.

The protein concentrations were determined by the method of Lowry *et al.* (1951).

Results

Chronic hypoxia. Although the body weight of the hypoxic animals was lower than that of the controls no changes were observed in liver weight to body weight ratios. Hemoglobin and hematocrit values increased about 50% during this period (Table I).

TABLE I Effect of chronic hypoxia on body weights, liver weight to body weight ratios, hemoglobin concentrations and hematocrit values.

	Control	Hypoxic (14 days, 380 mm Hg)
Body weight change (%)	$+12.9\pm0.80$ (15)	-3.6 ± 0.71 (15)
Liver wt. g/100 g body wt.	3.70 ± 0.80 (15)	3.70 ± 0.39 (15)
Hemoglobin, g/l	145.3 ± 6.2 (7)	210.7 ± 4.0^{b} (7)
Hematocrit	0.43 ± 0.02 (7)	0.66 ± 0.02^{c} (7)

Values are means ± S.D with the number of experiments in parenthesis. – the lowest weight (H) was observed on the 5th day in hypoxia. ^{b} – $p<0.001$ – $p<0.001$ (Student's test).

The ADP O ratio did not change during the chronic hypoxia, and as far as the cytochrome concentrations were concerned cytochromes aa and c were slightly lower but the difference between the control and hypoxic groups was not significant (Table II). The mitochondrial protein values, as calculated from the cytochrome aa concentrations, showed a decrease in the hypoxic group as compared with the control values (−15%).

In the mitochondrial enzyme activities, the chronic hypoxic period (2 weeks) resulted in an increase in succinate oxidase and succinate dehydrogenase activities. Cytochrome oxidase activity remained unchanged Malate dehydrogenase and glutamate dehydrogenase activities increased somewhat, but not significantly These results are shown in Table III When the control value in each experiment was marked as 100, succinate dehydrogenase activities in the hypoxic group increased by 31% in the total homogenate and by 23% in isolated mitochondria (K Fe(CN)$_6$ as an electron acceptor) The corresponding values for the dichloroindophenol method were 24% and 20%. The increase in succinate oxidase activity in the hypoxic group was 23%

Acute hypoxia During the hypoxic period of 5 hours succinate dehydrogenase, succinate oxidase and cytochrome oxidase activities increased (Table IV) Succinate oxidase activity in isolated mitochondria increased by 35% and cytochrome oxidase by 21%.

The lysosomal contamination was 15% and the microsomal contamination 3.5% in mitochondria. It was observed that more pure mitochondrial fraction could be obtained by washing several times with the isolation medium. However the isolation of this fraction takes time and may damage the functional integrity of the mitochondria.

TABLE II Comparison of mitochondrial cytochrome concentrations, ADP O ratios and protein values of altitude-exposed and control rats after weeks.

	Control	Hypoxic
Cyt aa$_3$	0.363 ± 0.101 (8)	0.330 ± 0.081 (8)
Cyt b	0.119 ± 0.026 (8)	0.116 ± 0.026 (8)
Cyt c	0.325 ± 0.064 (8)	0.314 ± 0.052 (8)
Cyt c$_1$	0.172 ± 0.067 (8)	0.146 ± 0.061 (8)
ADP O	1.89 ± 0.21 (3)	1.97 ± 0.17 (3)
Protein	53.1 ± 15.8 (3)	45.2 ± 17.8 (3)

Cytochrome concentrations are expressed as nmol/mg protein and protein values mg/g wet weight calculated from cytochrome aa concentrations in the total homogenate and in isolated mitochondria. Values are means ± S.D with the number of experiments in parenthesis.

…E III. Effect of chronic hypoxia (2 weeks) on succinate oxidase (SUCC OX), succinate dehydrogenase (SDH), cytochrome oxidase (CYT OX), malate dehydrogenase (MDH) and glutamate dehydrogenase (GDH) activities.

…yme	Fraction	Control	Hypoxic
[illegible]	Total homogenate	0.043 ± 0.006 (7)	0.057 ± 0.011 (7)[a]
[illegible]	Total homogenate	4.77 ± 1.01 (6)	6.05 ± 2.53 (6)
…C OX	Mitochondria	5.23 ± 0.92 (6)	6.35 ± 1.13 (6)[b]
…T OX	Mitochondria	18.4 ± 4.67 (5)	18.8 ± 4.75 (5)
…H	Mitochondria	2 617 ± 557 (8)	2 941 ± 544 (8)
…H	Mitochondria	122.6 ± 33.6 (7)	134.3 ± 42.2 (7)

…e activities are expressed as µl O_2/min/mg protein for cytochrome oxidase and succinate oxidase, units/…/mg protein for succinate dehydrogenase ($K_3Fe(CN)_6$ as an electron acceptor), nmol/min/mg (di-…orophenolindophenol as an electron acceptor), µmol/min/mg protein for malate dehydrogenase and glutamate …ydrogenase. Values are mean ± S.D. with the number of experiments in parenthesis. [a] $p < 0.02$, [b] $p < 0.1$ (Student's test).

Discussion

…cclimatization of the animals to low oxygen pressure for 2 weeks was indicated by the …anges in blood and body weight. No damage was caused to the mitochondria in these ex…riments, as the efficiency of oxidative phosphorylation did not change. These results …ree with previous studies (Strickland *et al* 1962, Ziegler 1967 Gold *et al* 1973). All these …bservations point to the fact that mitochondria are very resistant during altitude stress. … slight uncoupling of oxidation and phosphorylation has been observed, however after … day of exposure to 7 620 m (Nelson *et al*. 1967). It is probable that this kind of phenome…on is a transient change which is associated with the onset of altered conditions and …hich returns to normal once the adaptation process proves possible.

As far as the mitochondrial enzyme activities at altitudes are concerned the results of …his study show an increase in succinate dehydrogenase activities in both the acute and …hronic experiments. This reflects the dependence of the increase in activity on some kind …f activation and not on an increase in enzyme protein. It has been concluded that the in…crease in succinate dehydrogenase activity is the result of a structural change in an enzyme protein (Aithal and Ramasarma 1969). In this study cytochrome oxidase activity did not

TABLE IV Effect of acute hypoxia (5 h) on succinate oxidase (SUCC OX), cytochrome oxidase (CYT OX), succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and glutamate dehydrogenase (GDH) activities in isolated mitochondria.

Enzyme	Control	Hypoxic
SDH	0.224 ± 0.065 (8)	0.288 ± 0.048 (8)[a]
SUCC OX	4.47 ± 1.00 (5)	6.05 ± 0.91 (5)[b]
CYT OX	17.3 ± 4.23 (5)	20.9 ± 4.13 (5)[c]
MDH	1 772 ± 286 (5)	1 963 ± 499 (5)
GDH	79.9 ± 43.3 (5)	81.4 ± 38.1 (5)

The activities are expressed as µl O_2/min/mg protein for cytochrome oxidase and succinate oxidase, units/min/mg protein for succinate dehydrogenase ($K_3Fe(CN)_6$ as an electron acceptor) and µmol/min/mg protein for malate dehydrogenase and glutamate dehydrogenase. Values are mean ± S.D. with the number of experiments in parenthesis. [a] p 0.05, [b] 0.05, [c] $p < 0.1$ (Student's test).

change during the longer hypoxic period, though it increased during the acute experim These results point to permanent activation of the succinate dehydrogenating enzyme as a consequence of hypoxic stress and adaptation The transient increase in cytochrome dase activity may be part of the hypoxic reaction.

The exposure of rats to low atmospheric pressure for two weeks resulted in a decli the cytochromes This change was, however small and not statistically significant. The r tion of the cytochromes to hypoxia has not been resolved. In yeast cells it has been not that oxygen deficiency results in a decrease in cytochromes (Biggs and Linnane 19 Hallman (1971) has observed that the concentration of cytochromes $c+c_1$ does not incr normally when the environment of neonatal rats becomes hypoxic. On the other han has been concluded that moderate hypoxia may even cause an increase in cytochrome c centrations (Sherzer and Cascarano 1972) More studies especially in more severe hyp are needed. The corresponding phenomena of the heart muscle might be of special inte because of the importance of oxidative metabolism.

It may be concluded that moderate hypoxia leads to some change in the mitochond metabolism, but does not destroy the mitochondria The efficiency of oxidative phosp rylation in altitude-exposed rats remains the same as in the controls. The low mitochond protein values indicate that the mitochondrial population has decreased or the composi of the mitochondria has changed As to the increased enzyme activities in the hypoxic gr their significance remains unclear It is possible that this increased enzyme function in moderate hypoxia is an attempt to adapt the tissue to increased energy demands.

References

AITHAL, H N and T RAMASARMA, Activation of liver succinate dehydrogenase in rats exposed to h baric conditions. *Biochem. J* 1969 *115* 77–83

APPELMANS, F R. WATTIAUX and C. DEDUVE, Tissue fractionation studies. 5. The association of phosphatase with a special class of cytoplasmic granules rat liver *Biochem. J* 1955 *59* 438–445

BEAUFAY Y H D S BENDALL, P BAUDHUIN and C DEDUVE, Tissue fractionation studies. 12. Intrac lar distribution of some dehydrogenases, alkaline deoxyribonuclease nd iron in rat-liver tissue *Bioc J* 1959 *73* 623–628

BIGGS, D R. and A W LINNANE, The effect of oxygen on the composition and organization of the tron transport system of yeast. *Biochim. biophys Acta* (Amst.) 1963 *78* 785–788

BONNER, W D Succinic debydrogenase. In *M th in Enz I* (Eds. S. P Colowick and N O Kap Academic Press, New York and London, 1955 722–729

CHANCE, B. and G R. WILLIAMS, Respiratory enzymes oxidative phosphorylation II Difference spe *J biol. Chem.* 1955 *217* 395–407

CHANCE, B., Reaction of oxygen w th the respiratory chain cells and tissues. *J gen. Ph siol.* 1965 163–195

CRIDDLE, R. S. and G SCHATZ, Promitochondria of naerobically grown yeast. I Isolation and bioch cal properties. *Biochemistry* 1969 *8* 322 334

ERNSTER, L., R ZETTERSTRÖM and O LINDBERG, A method fo the determination of tracer phosphat biological material. *A ta chem. scand.* 1950. *4* 941 947

GLINSMANN W H nd J L E ERICSSON Observations on the subcellula rgan zation of hepatic pa chymal cells. II Evolution of reversible alterations induced by hypoxia. *Lab. Invest* 1966. *15* 76.

GOLD A J T F JOHNSON and L. C. COSTELLO, Effects of ltitude stress on mitochondrial funct *Amer J Physiol.* 1973 *224* 946–949

GREEN, D E., S. MII and P M KOHOUT Studies on the terminal lectron transport system. I Succ dehydrogenase *J biol. Chem* 1955 *217* 551 567

HALLMAN, M., Changes in mitochondrial respiratory chain proteins during perinatal development. Evidence of the importance of environmental oxygen tension. *Biochim. biophys. Acta* (Amst.) 1971 *253*, 360–372.

HALLMAN, M., P. MÄENPÄÄ and I. HASSINEN, Levels of cytochromes in heart, liver, kidney and brain in the developing rat. *Experientia* (Basel) 1972. *28*, 1408–1410.

HASSINEN, I. Hydrogen transfer into mitochondria in the metabolism of ethanol. II. Effect of disulfiram on the hydrogen and energy transfer. *Ann. Med. exp. Fenn.* 1967 *45*, 46–56.

[illegible], V. Enzyme activities of liver mitochondria from altitude exposed rats. *Acta physiol. scand.* 1974. *91* 12 A.

LEIGHTON, F., B. POOLE, H. BEAUFAY, P. BAUDHUIN, J. F. COFFEY, S. FOWLER and C. DE DUVE, The large-scale separation of peroxisomes, mitochondria, and lysosomes from the livers of rats injected with Triton WR 1339. *J. cell. Biol.* 1968. *37* 482–513.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, Protein measurement with the Folin Phenol reagent. *J. biol. Chem.* 1951 *193*, 265–275.

NELSON, B. D., B. HIGHMAN and P. D. ALTLAND, Oxidative phosphorylation during altitude acclimation in rats. *Amer. J. Physiol.* 1967 *213*, 1414–1418.

O'BRIEN, T. W. and G. F. KALF, Ribosomes from rat liver mitochondria. I. Isolation procedure and contamination studies. *J. biol. Chem.* 1967 *242*, 2172–2179.

PARK, C. D., L. MELA, R. WHARTON, J. REILLY, P. FISHBEIN and E. ABERDEEN, Cardiac mitochondrial activity in acute and chronic cyanosis. *J. Surg. Res.* 1973, *14* 139–146.

RABINOWITZ, M., V. ASCHENBRENNER, R. ALBIN, N. J. GROSS, R. ZAK and K. G. NAIR, Synthesis and turnover of heart mitochondria in normal, hypertrophied and hypoxic rat. In: *Cardiac Hypertrophy* (Ed. N. Alpert). New York: Academic Press. 1971 283–299.

SANDERS, A. P., D. M. HALE and A. T. MILLER, Jr. Some effects of hypoxia on respiratory metabolism and protein synthesis in rat tissues. *Amer. J. Physiol.* 1965. *209* 443–446.

SCHOLLMEYER, P. and M. KLINGENBERG, Über den Cytochrom-Gehalt tierischer Gewebe. *Biochem. Z.* 1962. *335* 426–439.

[illegible], H. G. and J. CASCARANO, Mitochondrial alterations in heart, liver and kidney of altitude-acclimated rats. *Amer. J. Physiol.* 1972. *223*, 632–636.

SIEGEL, L. and S. ENGLARD, Beef heart malic dehydrogenases. I. Properties of the enzymes purified from extracts of acetone-dried powders. *Biochim. biophys. Acta* (Amst.) 1961 *54*, 67–76.

STRICKLAND, E. H., F. E. ACKERMAN and A. ANTHONY, Respiration and phosphorylation in liver and heart mitochondria from altitude-exposed rats. *J. appl. Physiol.* 1962. *17* 535–538.

UMBREIT, W. W., R. H. BURRIS and J. F. STAUFFER, *Manometric techniques*. Burgess Publishing company 1964 159–167.

VANNESTE, W. H., Molecular proportion of the fixed cytochrome components of the respiratory chain of Keilin-Hartree particles and beef heart mitochondria. *Biochim. biophys. Acta* (Amst.) 1966. *113* 175–178.

[illegible], F. D., Respiratory and phosphorylative responses to hypoxia and food restriction. *Amer. J. Physiol.* 1967 *212* 197–202.

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References

AITHAL, H N and T RAMASARMA, Activation of liver succinate dehydrogenase i rats exposed to hy baric conditions. *Biochem J* 1969 *115* 77–83

APPELMANS, F R. WATTIAUX and C. DEDUVE, *Tissue fractionation studies. 5. The association of* phosphatase with a special class of cytoplasmic gra ules rat liver *Biochem J* 1955 *59* 438–445.

BEAUFAY Y H D S. BENDALL, P B UDHUIN and C. DEDUVE, Tissue fractionation studies. 12. Intrace lar distribution f some dehydrogenases, alkaline deoxyribonuclease and ron in rat-liver tissue. *Bioch J* 1959 *73* 623–628.

BIGGS, D R. and A W LINNANE, The effect of oxygen o the composition and organization of the e tron transport system of yeast. *Biochim. biophys. Acta* (Amst.) 1963. *78* 785–788.

BONNER, W D Succinic dehydrogenase. I *Meth in Enz 1* (Eds. S. P Colowick and N. O. Kapl Academic Press, New York and London, 1955 723–729

CHANCE, B. and G R. WILLIAMS, Respiratory enzymes in o idati e phosphorylation II Difference spec *J biol. Chem.* 1955 *217* 395–407

CHANCE, B., Reaction of oxygen with the respiratory chain in cells and tissues. *J gen Physiol* 1965. 163–195

CRIDDLE, R. S. and G SCHATZ, *Promitochondria of anaerobically grown yeast. I Isolation and bioche* cal properties. *Biochemistry* 1969 *8* 322 334

ERNSTER, L., R. ZETTERSTRÖM and O LINDBERG, A method for the determination of tracer phosphat biological material. *Acta chem. scand* 1950. *4* 941 947

GLDERMANN, W H and I L E ERICSSON, Observations on the subcellular rganization of hepatic par chymal cells. II Evolution of reversible alterations induced by hypoxia. *Lab Invest* 1966. *15* 762 '

GOLD, A. J T F JOHNSON and L. C COSTELLO, Effects of ltitude tress mitochondrial funct *Amer J Physiol.* 1973 *224* 946–949

GREEN D E., S. MII nd P M KOHOUT Studies on the terminal electron transport system. I Succ dehydrogenase. *J biol. Chem.* 1955. *217* 551 567

re (MAP), sampling of arterial blood and venous infusions respectively Arterial P_{O_2}, P_{CO_2} and pH determined. Details of the procedures have been presented previously (Alm 1975). ce an additional aim of the experiments was to determine the effect of topical application of 1 pherine on ocular blood flow the animals were kept under pentobarbital anesthesia for varying times to blood flow determinations. Thus 3 monkeys were kept under anesthesia for 1 h, 3 for 4 h and 1 h. A thoracotomy was then performed and the radioactively labelled microspheres ere injected into ft ventricle of the heart. Ideally the spheres should act as non-recirculating blood flow indicator and re trapped within terminal arterioles, or capillaries, in proportion t blood flow Microspheres of different sizes labelled with ^{85}Sr and ^{141}Cr respectively ere used. According t the manufacturer Company St Paul, Minnesota) the diameters of the spheres ere 15 ± 5 µm and 35 ± 5 µm respectively d of 1 percent suspensions of each sphere size were mixed and injected. The specific activity for both re types was 10 mCi/g. At the start of the intracardiac injection and for the following 60 blood was ted from cannulated femoral artery to serve as reference flow after which the animals were killed injections of KCl. Tissue samples were taken, blood and tissue samples were weighed and the activity was determined by gammaspectrometry Regional blood flow estimates could then be calculated for both sphere sizes by dividing the activity of the tissue sample by the activity per mg blood flow was calculated for the reference flow Such determinations were made for lung, ocular tissues and various ns of the brain. Both lungs were removed, weighed and minced and the radioactivity of 4 representative ples was determined to estimate the overall leakage of the two sphere sizes through the systemic vascular . Most of the pia was removed from the cerebral hemispheres, and the radioactivity of the pia was mined, as rule as double samples. *Double samples were taken from grey and white matter of the* tal, temporal and occipital lobes. 4 samples were taken from the corpus callosum, and the choroid us was taken as one sample. Values obtained for ocular blood flow will be presented elsewhere.

Results

the time of injection of the microspheres the following data were obtained, mean ± S.E. 7). MAP 121 ± 7 cm H_2O arterial P_{O_2}, P_{CO_2} and pH 78 ± 5 mmHg, 33.0 ± 2.0 mmHg d 7.53 ± 0.03 units respectively Blood flow" to the lung was 29 ± 9 g/min per 100 g tissue lculated for the 15 µm spheres and 32 ± 9 for the 35 µm spheres.

Table I presents the blood flow values obtained with the two sphere sizes in various parts the brain. Flow values for grey matter are higher with 35 µm spheres than with 15 µm heres while the reverse happens for white matter including corpus callosum. No differ ce is seen for the choroid plexus. With 15 µm spheres there were no significant differences flow through the various lobes for either grey or white matter

For both sphere sizes a certain amount of spheres were trapped in extracerebral pial rterioles, and thus erroneously excluded in rCBF-determinations. The number of spheres apped in pial arterioles corresponds to a blood flow of 37 ± 7 (= 7) and 1756 ± 486 (n = 7) /min per 100 g pia for 15 µm and 35 µm spheres respectively Thus with 35 µm spheres, on weight basis, almost 30 times as many spheres are trapped in pial arterioles as in grey matter while for 15 µm spheres this ratio is about 1

To evaluate the significance of the difference in flow obtained with the two sphere sizes n the various tissues, the mean value of the sample differences (flow calculated from 35 µm spheres)—(flow calculated from 15 µm spheres) is presented in Table II. The differences were significant for both grey and white matter including corpus callosum. A similar calculation for the pia also resulted in a significant difference, 1756 ± 411 (n = 11) g/min per 100 g pia (p 0.005).

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Radioactively Labelled Microspheres in Regional Cerebral Blood Flow Determinations
A study on monkeys with 15 and 35 μm spheres

By

ALBERT ALM

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Abstract

ALM, A. *Radioactively labelled microspheres in regional cerebral blood flow determina… A study on monkeys with 15 and 35 μm spheres* Acta physiol scand. 1975 95 60–…

15 μm and 35 μm radioactive microspheres, differently labelled were used simultaneously for … determinations in monkeys. Flow values calculated from data for 35 μm spheres were 27±7 g/m… 100 g higher for grey matter and 10±3 g/min per 100 g lower for white matter than flow values calc… from 15 μm spheres, while both sphere sizes resulted in similar values for the choroid plexus. A… number of 35 μm spheres were trapped in extracerebral pial arterioles. It is concluded that entrapm… relatively large arterioles of the 35 μm spheres explains the differences in flow values, and that this inval… the use of 35 μm spheres, and larger in rCBF-determinations.

The use of intracardiac injections of radioactively labelled microspheres has made it p… ble to make simultaneous determinations of blood flow through various parts of the … (Rudolph and Heymann 1967) The method has been used for determinations of b… flow through whole organs and for determinations of regional blood flow within or… However studies on cardiac muscle (Domenech *et al* 1969 Yipintsol *et al* 1973 and … *et al* 1974) and kidney cortex (Katz *et al* 1971) have shown that localization of sp… within an organ may not be determined only by regional blood flow Other factors … affect sphere distribution such as axial streaming of spheres, and spheres that either e… through the capillary bed or become trapped in arterioles proximal to the tissue sa… These factors are influenced by the size of the injected spheres. Thus in the present … spheres of two sizes were injected simultaneously to evaluate to what extent these fa… influence regional cerebral flow blood (rCBF) determinations in monkeys.

Methods

7 monkeys of both sexes, 6 cynomolgus (*Macaca irus*) and one vervet (*Cercopithecus aethiops*), … between 2.0 and 3.3 kg were used. The animals were anesthetized, tracheotomized and artificially venti… Both femoral arteries and one femoral vein were cannulated for measurements of mean arterial …

eres in this respect. Large spheres may become trapped in too large arterioles not in-ded in the sample, and axial streaming may cause erroneous distribution of spheres with-i tissue. The degree of axial streaming increases with sphere size (Phibbs and Dong 1971) l the error introduced in sphere distribution by this rheological property of the spheres y also be assumed to increase with sphere size. If these errors are of minor importance eres of two sizes injected simultaneously should result in similar flow values. If not, it is ely that the larger spheres are in error provided that significant leakage of the smaller eres has not taken place.

The difference between flow values calculated for 15 and 35 μm spheres respectively in e present study (Table II) emphasizes that sphere size influences calculated rCBF-values. e difference can not be attributed to disturbances in local circulation caused by the icrospheres since the two sphere sizes were mixed before injection. The assumption that e larger spheres are in error is supported by the high content of 35 μm spheres within al vessels, which ideally should contain practically no spheres at all. Trapping of spheres ithin pial vessels can not be explained by axial streaming, and, with known data for vascular iameters within the brain, this suggests that trapping of 35 μm spheres within large arterioles the main obstacle that makes this sphere size, and larger unsuitable for rCBF-deter inations. A similar error caused by large spheres in regional blood flow determinations ave been reported by Katz *et al.* (1971) who found that 80 μm spheres resulted in errone usly high blood flow values for the deeper layers of the kidney cortex due to entrapment i proximal parts of the arteriole. The number of 15 μm spheres trapped within pial vessels n the present study shows that this source of error is unlikely to invalidate 15 μm spheres, r smaller

The present study permits no definite conclusions concerning the significance of a possible error due to axial streaming of 15 μm spheres. Axial streaming in the large transcerebral arterioles may favour white matter on behalf of grey matter In the present study the flow through grey matter is somewhat low This may be due to the prolonged anesthesia. Barbiturate anesthesia reduces cortical blood flow (Landau *et al.* 1955) In previous reports on rCBF-studies on monkeys, anesthetized for a much shorter time, 15 μm spheres resulted in mean values for white matter of 25–28 and for grey matter of 56 g/min per 100 g tissue (Alm and Bill 1973 Alm 1975). In the baboon James, Millar and Purves (1969) found flow values of 15–23 and 62–83 ml/min per 100 g tissue for white and grey matter respectively by intraarterial injections of ¹³³Xenon. The discrepancy between the results obtained by the two methods may have several explanations; difference in anesthetic depth, an inappropriate comparison since the anatomical localization of the flows measured by the two methods may not be identical, or axial streaming of the microspheres. That part of the discrepancy is explained by the latter possibility seems likely However studies on kidney cortex and cardiac muscle suggest that errors due to axial streaming of small spheres are not large enough to invalidate these in regional blood flow determinations. Thus in the dog kidney cortex 15 μm spheres and radiolabelled frog red blood cells resulted in similar values for egional blood flow (Baehler *et al.* 1973). In cardiac muscle Yipintsoi *et al.* (1973) and Utley *et al.* (1974) found that larger spheres exaggerated flow through any area with more than average blood flow usually subendocardial areas, as judged by smaller spheres

TABLE I Calculated blood flow through various parts of the brain in g/min per 100 g tissue. Flow va calculated from 15 µm spheres and from 35 µm spheres are presented. Mean ± S E, 7 animal

Tissue	15 µm spheres	35 µm spheres
Grey matter		
Frontal lobe	35±6	57±10
Temporal lobe	28±5	33±6
Occipital lobe	40±6	92±19
White matter		
Frontal lobe	18±2	11±3
Temporal lobe	21±3	8±2
Occipital lobe	22±2	15±7
Corpus callosum	15±2	7±2
Choroid plexus	312±44	316±92

Discussion

The pial vessels provide a vascular net supplying grey and white matter of the cerebral he spheres through centripetal branches. There is no strict division into grey and white ma arterial vessels although a certain pattern exists. Thus superficial layers of grey matter supplied by short cortical arterioles that do not enter white matter while deeper layers supplied by cortical arterioles which sometimes also give branches to white matter Wh matter is mainly supplied by larger transcerebral arterioles which, however give branc also to deeper layers of grey matter (Campbell 1938 Saunders, Feindel and Carvalho 196 The diameters of these vessels are of obvious importance for the choice of sphere size. P arterioles may be as small as 15–20 µm before entering brain substance (Nielsen and Owm 1967 Dahl 1973). The diameters of the larger transcerebral arterioles range from 30 to µm as they pass through grey matter (Saunders and Bell 1971) Since brain capillaries in the order of 6 to 8 µm passage of 15 µm spheres through the cerebral vascular bed m be negligible. Spheres that pass through the systemic vascular bed are likely to beco trapped within the lung. The similarity of the flow values calculated for the lung for 15 a 35 µm spheres in the present study indicates that the difference between the two sphere si in this respect is not significant.

The use of microspheres in studies on regional blood flow implies that the spheres trapped within small tissue samples in proportion to blood flow Apart from passage small spheres through the capillary bed two other possible errors might invalidate t

TABLE II Differences between blood flow values calculated from the two sphere sizes n samples fr various parts of the brain, and significance levels (Student s t test). The difference (flow c culated from 35 µm spheres)—(flow calculated from 15 µm spheres) was used. Mean ±S (n) = number of samples.

Tissue	Difference (g/min per 100 g tissue)	Significance level
Grey matter	27±7 (42)	$p<0.001$
White matter	− 10±3 (42)	$p<0.005$
Corpus callosum	− 8±2 (28)	$p<0.005$

lph, A. M. and M. A. Heymann, The circulation in the fetus in utero. Methods for studying distribution of blood flow cardiac output and organ blood flow Circulat. Res. 1967 21 163–184.
ders, R L. de C. H. and M. A. Bell, X-ray macroscopy and histochemistry of the human cerebral od vessels. J Neurosurg 1971 35 128–140.
ders, R. L. de C. H W H. Feindel and V R. Car alho, X-ray macroscopy of the blood vessels the human brain. Med. Biol. Illustr 1965. 15 108–122.
r J E. L. Carlson, J I. E. Hoffman, H. M. Martinez and G D Buckberg, Total and regional ocardial blood flow measurements with 25 μm, 15 μm, 9 μm, and filtered 1 10 μm diameter microspheres and antipyrine in dogs and sheep. Circulat Res. 1974. 34 391–405.
oso, T W A. Dobbs, Jr, P D Scanlon, T J Knapp and J B. Bassingthwaighte, Regional distribution of diffusible tracers and carbonized microspheres in the left ventricle of isolated dogs heart. culat. Res. 1973. 33 573–587

and by diffusable tracers such as antipyrine. This unfavourable comparison was found between 15 μm and 9 μm spheres, but the difference was usually less than 10 per cent (U et al 1974) Limitations of antipyrine in regional blood flow determinations, discussed these latter investigators, led them to conclude that 9 μm spheres are probably the b indicator for regional blood flow determinations in cardiac muscle.

The microspheres offer obvious advantages in rCBF-determinations. The possibility an exact anatomical localization with high spatial resolution, without intervening surg trauma prior to flow determinations, may also be obtained with a diffusable tracer such ¹⁴C-ethanol (Eklöf *et al* 1974) However unlike diffusable tracers, differently label microspheres permit repeated flow determinations, *e.g* before and after a change in s physiological parameter Thus radioactively labelled microspheres may be a valuable met for rCBF-determinations, but more information concerning the significance of axial stre ing in cerebral vessels is needed A comparison between flow values obtained with sin taneously injected 9 μm and 15 μm spheres may provide such information.

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References

ALM A The effect of stim lati of the cervical sympathetic chain on regional cerebral blood flow monkeys. A study with radioactively labelled microspheres. *Acta physiol scand* 1975. *93* 483–489

ALM, A. and A. BILL, Ocular nd optic nerve blood flow at normal and increased intraocular press in monkeys (Macaca irus) study with radioactively labelled microspheres including flow determinati in brain and some other tissues. *Exp Eye Res.* 1973 *15* 15–29

BAEHLER, R W A. J CATANZARO, J H STEIN and W HUNTER, The radiolabelled frog red blood A new marker of cortical blood flow distribution i the kidney of the dog. *Circulat Res.* 1973. *32*. 718–

CAMPBELL, C. P The vascular architecture of the cat's brain. A study by vital injection. *Assoc. Res. Dis. Proc* 1938. *18* 69–93

DAHL, E., The innervation of the cerebral arteries. *J Anat* 1973 *115* 53–63

DOMENECH, R. J J I E. HOFFMAN, M I M NOBLE K. B. SAUNDERS, J R. HENSON and S. SUBIJA Total and regional coronary blood flow measured by radioactive microspheres conscious and anes tized dogs. *Circulat Res.* 1969 *25* 581 596.

EKLÖF B., N A. LASSEN, L. NILSSON, K. NORBERG, B. K. SIESJÖ and P TORLÖF Regional cerebral bl flow in the rat measured by the tissue sampling technique critical evaluation using four indica C¹⁴-antipyrine, C¹ -ethanol, H water nd Xenon. *A t ph siol. scand* 1974 *91* 1 10.

JAMES, I. M R. A. MILLAR and M J PURVES, Observations on the extrinsic neural control of cere blood flow in the baboon. *Circulat Res* 1969 *25* 77 93

KATZ, M A., R. C. BLANTZ, F C. RECTOR, J nd D W SELDIN, Measurement of intrarenal blood fl I Analysis of microsphere method. *Amer J Physiol* 1971 *220* 1903–1913

LANDAU W M W H FREYGANG, J L P ROLAND, L SOKOLOFF and S. S KETY The local circulat of the living brain values in the nanesthetized nd nesthetized cat. *Trans. Amer neurol Ass.* 1 *80* 125–129

NIELSEN, K. C. and C. OWMAN, Adrenergic innervation of pial rteries related to the circle of Willis in cat. *Brain Res.* 1967 *6* 773–776.

PHIBBS, R. H and L. DONG, Nonuniform distribution of microspheres in blood flowing through a mediu size artery *Can. J Physiol Pharm.* 1970 *48* 415–421

terase since a normal end-plate can be formed on an area of muscle that was never inner vated previously (Hoffman 1951). The appearance of these receptors is therefore directly dependent on the presence of the motor nerve. Once formed, however junctional receptors are intrinsically stable: they persist for some time following denervation. Hartzell and Fambrough (1972) noted approximately normal junctional receptor density after 1 2 weeks of denervation. Similarly Lømo and Rosenthal (1972) reported persistent, normal peaks of high ACh sensitivity at denervated end-plates when muscles were directly stimulated for 2 weeks. On muscle fibres innervated by a foreign nerve at a different site some of the old denervated end-plates remain sensitive to ACh for over a year (Frank *et al* 1975).

These observations suggest that the long-term integrity of an end-plate, once established, might therefore be maintained by the metabolism of a normally active muscle rather than through some "trophic" influence of the nerve. In an attempt to discriminate among the various possibilities we studied the stability of junctional receptors at denervated end-plates on muscles in three different experimental situations denervated muscles, denervated muscles kept active by direct electrical stimulation, and muscles innervated by a foreign nerve at some distance from the old denervated end-plates. The number of receptors was assessed by measuring the amount of [125I] -bungarotoxin bound to old end-plates. This toxin binds specifically and nearly irreversibly to ACh receptors (Miledi and Potter 1971 Berg *et al.* 1972) and the high specific radioactivity of the toxin used in these experiments enabled us to quantitatively measure the ACh receptors at individual end-plates.

Methods

Experimental preparations

The end-plates studied in these experiments were on soleus muscle fibres from 200–300 g Wistar albino male rats. Operations were performed under Nembutal anaesthesia. For each animal, one leg was operated and the other was used as control. 3 types of experimental material were prepared.

Denervated-stimulated muscles. A pair of stimulating electrodes was implanted in the adjacent musculature so that they were not in direct contact with the soleus muscle. Current between the two electrodes was transverse to the muscle. The electrodes were platinum sheets covered with medical Silastic (738 RTV Dow Corning) which was scraped away to expose few mm² of metal on the side facing the soleus muscle. Solder connections were covered with Silastic. Stimulation began the same day as denervation (5–7 mm resection of the sciatic nerve in the popliteal fossa) and consisted of trains of 100 pulses of 3 ms duration at 10 Hz repeated every 12 The stimulus polarity was reversed between each train, and intensity was adjusted to give an easily visible and palpable contraction of the leg musculature. Effectiveness of the stimulation was checked daily and was confirmed at the end of the experiment by the absence of appreciable α-bungarotoxin binding to extra-junctional membrane (see below). Absence of reinnervation was also confirmed just before incubation in the toxin by stimulation of the distal end of the cut soleus nerve as it entered the muscle.

Denervated muscle. The muscle was denervated as described above. Stimulation was begun 10–14 days before the muscle was examined in order to reduce ACh sensitivity and toxin binding outside the end-plate region. In experiments lasting less than 3 weeks, no electrical stimulation was used. Absence of reinnervation was confirmed in each case.

Cross-innervated muscles. Soleus muscles were cross-innervated with the superficial branch of the fibular nerve. The branch was dissected, cut distally and transplanted onto the proximal dorsal surface of the soleus muscle and held in place with fibrin clot. About two weeks later the tibial nerve (which contains the soleus motor axons) was cut in the popliteal fossa and implanted into adjacent biceps muscle to prevent reinnervation of the soleus muscle. The extent of cross-innervation and absence of reinnervation was checked before incubation in the toxin.

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Cholinergic Receptors at Denervated Mammalian Motor End Plates

By

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Abstract

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The number of acetylcholine receptors t normal and denervated end-plates in rat soleus muscles wa studied using the binding of [^{125}I] α bungarotoxin as a quantitative assay Normal end-plates bound several thousand times as much toxin as equal areas of extra-synaptic muscle membrane. After short term denervation (up to 2.4 weeks) the extrajunctional binding increased, but there was no change specific bindi g to the motor end-plate. Denervation for longer periods (up to 7 weeks) reduced binding sites at the end-plate by up to 60–70%. Direct electrical stimulation of these muscles for the same period of denervation did not prevent the loss of junctional bindi g sites even though it was adequate to bolish the increase i extrajunctional toxin bind ng. In contrast, denervated end-plates on muscle fibres cross-innervated by a foreign nerve at a distant location continued to bind normal amounts of toxin for over four months.

Embryonic mammalian skeletal muscle fibres are sensitive to acetylcholine (ACh) along their entire length Shortly after birth, however this sensitivity becomes restricted t the small area of synaptic contact between nerve and muscle (Diamond and Miledi 196 When impulse activity in the motor nerve is blocked or the nerve itself is destroyed, ACh sensitivity again appears outside the synaptic region (Axelsson and Thesleff 1959 Lømo and Rosenthal 1972).

The development of high extra-synaptic sensitivity in the absence of a functional nerve supply might suggest that the nerve directly determines the distribution of ACh sensitivity However Lømo and Rosenthal (1972) have shown that direct electrical stimulation of denervated muscle fibre also produces and maintains a low extra-junctional sensitivity Under normal conditions the electrical and/or mechanical activity of the muscle may therefore be sufficient to account for the absence of sensitivity outside the synaptic region

In the synaptic region itself the formation and regulation of ACh receptors is only poorly understood. Unlike extra-junctional receptors, these receptors do not disappear during normal muscle activity Initially a nerve can induce the appearance of junctional receptors and ACh

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Results

...tylcholine esterase staining of denervated end-plates

...l three types of denervated end-plates were adequately visualized with the acetylthio-...oline stain, but the distribution and intensity of the reaction product were different than ...: normal innervated end-plates. Representative examples are shown in Plate 1 an in-...rvated end-plate is included for comparison. Denervated end-plates on muscle fibres ...at had been directly stimulated for up to 6 weeks from the time of denervation (Plate 1 B) ...re similar to those on fibres denervated for the same length of time but stimulated only ...r the final 1 1/2 weeks (Plate 1 C). The stain was as dense as at a normal end-plate (Plate ... D) but the end-plates appeared to be much narrower. These esterase-stained end-plates ...e similar to those on denervated unstimulated fibres.

The staining of a denervated end-plate on a muscle fibre innervated elsewhere by another ...erve is shown in Plate 1 A. Even after several months of denervation (and cross-innervation) ...e old end-plate stained well. Frequently there were long filamentous strands of reaction ...roduct extending from the end-plate region and often the intensity of the stain was less ...ense than normal.

...inding of labelled toxin

...The binding of [^{125}I] α-bungarotoxin to end-plates and extrajunctional areas on a control ...nd a denervated muscle is shown in Fig. 1. In normal muscles toxin binding was more than ...0 times higher in the end-plate region than in the extra-junctional area. The actual end-...late occupies only small fraction of the membrane in our "end-plate regions". If one ...ssumes that toxin binding is normally confined to the synaptic area only (Hartzell and ...Fambrough 1972), the end-plate binds several thousand times more toxin than equal areas ...of non-endplate regions. The amount of toxin bound per end-plate was comparable to that ...found by other groups (Miledi and Potter 1971 Berg *et al.* 1972, Hartzell and Fambrough ...1972). In order to investigate the effect of denervation alone, we measured toxin binding to ...wo muscles denervated for a short time (1 4 and 2.4 weeks) that had not been stimulated ...at all. Fig. 1 illustrates the results from one of these muscles. Assuming that the extra-...junctional binding is approximately uniform along the length of a denervated fibre we ...calculated end-plate specific toxin binding by subtracting out the binding to extra-...junctional parts of the fibres in a manner similar to that used by Guth, Albers and Brown ...1964) and Hall (1973) for cholinesterase. This assumption is qualitatively supported by ...autoradiographs prepared by Hartzell and Fambrough (1972, Fig. 1 B) and by binding data ...from Miledi and Potter (1971 Fig. 4) but apparently has not been rigorously tested. With ...this reservation, the binding to these end-plates (denervated 1 4 and 2.4 weeks) is not signifi-...cantly different from the controls (see Fig. 4), in accord with earlier results (Hartzell and ...Fambrough 1972, Lømo and Rosenthal 1972).

To assess the long-term effects of denervation we stimulated the muscles for the final ...1 1/2 weeks in order to abolish the high toxin binding to extrajunctional areas of the fibres. ...As shown in Fig. 2 the binding of [^{125}I] α-bungarotoxin to end-plates on the denervated ...muscle fibres was reduced after period of six weeks whether or not the muscles were chroni-

Fibres innervated by the foreign nerve were of normal diameter while those that ere not cros vated were extremely atrophic. This provided an unambiguous identification of cross-innervated Only end plates on normal sized fibres in regions of the muscle known to be cross-innervated were for analysis. The absence of extra junctional toxin binding provided further assurance that these were functionally innervated (see below). It was also important to verify that the foreign nerve h reinnervated the old soleus end plates. This was confirmed by looking for any nerve fibres in soleus end-plate region, both in the whole muscle with a dissecting microscope and in teased fibr arations examined with Nomarski optics. The esterase stain facilitated location of the end-plates i preparations. Two of six cross-innervated muscles appeared to have some foreign reinnervation end-plates and were not included in the results.

Preparation f [^{125}I] α-bungarotoxin

Purified α-bu garotoxin (supplied by Miami Serpentarium) was assayed and migrated as a singl in 10 % polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS). The (20 μg) was labelled with carrier-free $Na^{125}I$ (3 mCi) using the chloramine T method (Gree Hunter and Glover 1963). Bovine serum albumin was added after the iodination to a final concen of 0.2 %. After separation of the iodinated toxin from free ^{125}iodide by gelfiltration on Sephade it was diluted in Kreb s solution (final concentrations, mM Na^+ 162, K^+ 5; Ca 2 Mg^{2+} 1 H 1; HCO_3^- 24 Cl 148 glucose, 11) and stored in 15 one ml aliquots at −20°C.

The addition of albumin t the toxin solutions complicated the measurement of specific activi an estimate was made in the following way: Each aliquot of toxin had the same biological activity μg/ml solution of unlabelled toxin as determined by the rate of blockade of neuromuscular trans in an isolated rat s diaphragm muscle (~45 min). This suggests that at least 75 % of the initial tox recovered, and with this assumption the specific activity of 3 batches of labelled toxin varied b 4 and 14 10^5 Ci/mole.

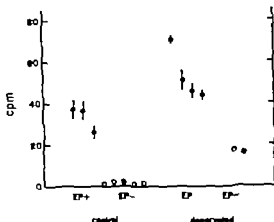
SDS polyacrylamide gel electrophoresis (10 %) of the labelled toxin showed that more than 9 the total radioactivity migrated as a single peak with a mobility corresponding to that of the unl toxin. There was no detectable loss of binding activity after 10 weeks of storage.

Binding of toxin to muscles

Experimental and control soleus muscles were isolated under ether anaesthesia, dissected free o nective tissue, nd incubated together in 1 ml of Kreb s solution containi g approximately 1 μg o for 2 h at room temperature. The solution was continually aerated with 5 % CO_2 and 95 % O_2. Preli experiments indicated that binding of toxin to superficial end plates was complete in one hour. The m were washed overnight in a small volume of oxygenated Kreb solution at 4°C that was continuo placed at 2 ml/min. After a short prefixation (10 m) i 1 % glutaraldehyde in Kreb s solution, the stained for ACh-esterase (Karnovsky 1964) and placed overnight in a 4 aldehyde fixative.

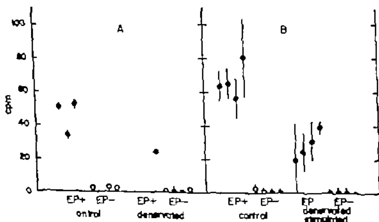
Small groups of superficial muscle f bres approximately 1 mm long and containing 1 15 f bre teased out, and the number of stained end-plates were counted nd compared with the number of fibres. The fibres were then dissolved in 0.5 ml of formic acid nd the radioactivity was measure Pack rd gamma scintillation spectrometer (50 cpm background 90 % co nting efficiency). N end-plates from the unoperated contralateral soleus muscle were always used to determine control b levels, although the vari tions in amou t of toxin bind ng to normal end-plates from different were small. The results are presented as cpm per end-plate, corrected for isotopic decay of iodine b corrected for extra-junctional to in binding (see below).

Similar lengths of fibres without end plates were used to measure non-end-plate binding. Bind extra-junctional regions included the bindi g to extra-junctional ACh receptors and to other site some affinity for the toxin. It therefore represents uppe limit on background binding. In norma cross-innervated muscles, nd in denervated muscles that had been adequately stimulated extra-junc binding was low enough (Fig. 2 and 3) so that it did not interfere with the measurement of binding end-plates. Denervated-unstimulated muscles, however alw ys showed marked binding of toxin o the end-plate region (Fig. 1). Toxin binding to non-end-plat regions thus provided a useful contr the adequacy of electrical stimulation. Two stimulated muscles had a higher the ormal non-end binding and were not included in the results because they were probably not adequately stimulated. as for all the others the amount of toxin bound per mm of fibre length t least 10 times smalle that associated with a single normal end-plate.



...nding of ¹²⁵I-α-bungarotoxin to normal soleus muscle (left) and soleus muscle denervated 10 ...ys (right). The filled circles in this figure and in Fig. 2 and 3 show the amount of bound ... end-plates on one mm lengths of muscle fibres (EP+). The open circles show the amount of ...xin per 1 mm length of muscle fibre without end-plate (EP−). The increased binding to end-plate ...in the denervated muscle is probably explained by the high extra-junctional binding in this muscle. ...ars here and in Fig. 2 and 3 indicate ±2 S.E. associated with the determination of radioactivity ...group of end plates. The 5 points with the largest error bars are groups that contained 2 or ...ibres each. Control muscles in this and subsequent figures are the contralateral unoperated ...muscles.

...stimulated for the entire period of denervation. The results from two binding experi-...m chronically denervated muscles are presented. In each case, the amounts of toxin ...ssed as cpm/end-plate) bound to normal and denervated end-plates is shown as well ...background levels for extrajunctional portions of normal and denervated fibres. Error



...2. Toxin binding to denervated end-plates on two non-innervated muscles. Both experimental muscles ...been denervated 6 weeks earlier. In A, the denervated muscle was electrically stimulated only for ...last 12 days, while in B, the muscle was stimulated continually for the entire period. Neither muscle ... significant number of extra-junctional binding sites but in both muscles the denervated end-plates ...ed only half the normal amount of toxin. Filled circles in A represent 5–10 end-plates each, in B all ...ups except the one with the smallest error bar contained 1–3 end-plates.

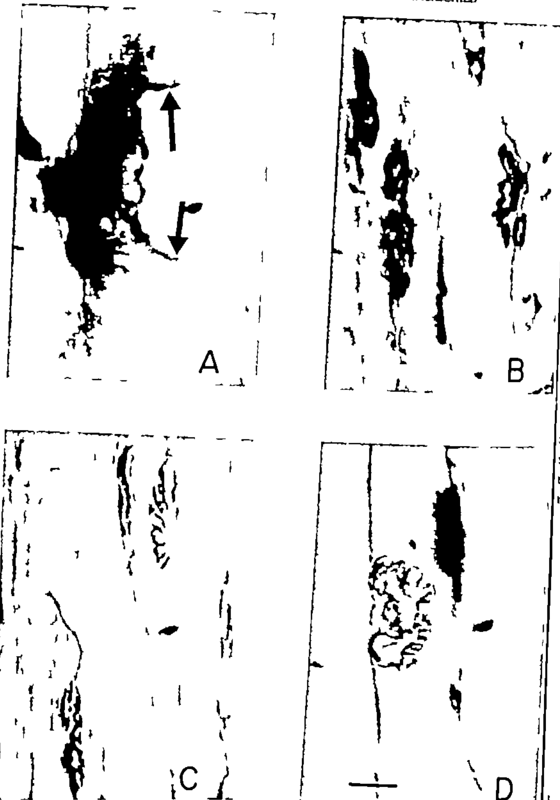


Plate 1 End plates stained for ACh-esterase from soleus muscles that had been ncubated ¹²⁵I-α-bungaro toxin. A. Denervated end-plate on muscle f bre that had been denervated and cross-innervated fou months earlier Arrow marks f lamentous strands of reaction product extending from the end-plate B Deners ted end-plates on f bres that had been deners ted and chronically stim lated for six weeks. C A in B, but electrical stimulation was ly for the final 12 days of the si week denervation period. D A normal innervated end-plate from an unoperated soleus muscle. The marker 20 μm all pic

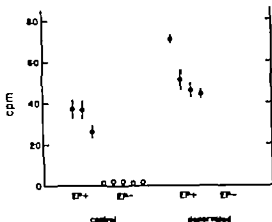


Fig. 1. Binding of ^{125}I-α-bungarotoxin to normal soleus muscle (left) and soleus muscle denervated 10 days earlier (right). The filled circles in this figure and in Fig. 2 and 3 show the amount of bound toxin per end-plate on one mm lengths of muscle fibres (EP+). The open circles show the amount of bound toxin per 1 mm length of muscle fibre without end-plate (EP−). The increased binding to end-plate regions in the denervated muscle is probably explained by the high extra-junctional binding in this muscle. Error bars here and in Fig. 2 and 3 indicate ±2 S.E. associated with the determination of radioactivity of each group of end-plates. The 5 points with the largest error bars are groups that contained 2 or 3 end-plates each. Control muscles in this and subsequent figures are the contralateral unoperated soleus muscles.

cally stimulated for the entire period of denervation. The results from two binding experiments in chronically denervated muscles are presented. In each case, the amounts of toxin (expressed as cpm/end-plate) bound to normal and denervated end-plates is shown as well as the background levels for extrajunctional portions of normal and denervated fibres. Error

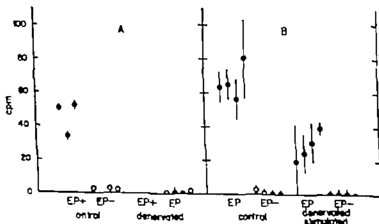
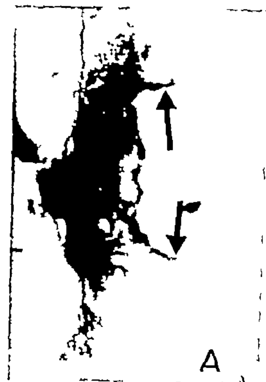
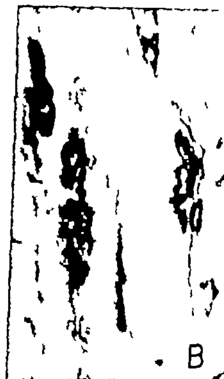


Fig. 2. Toxin binding to denervated end plates on two non-innervated muscles. Both experimental muscles had been denervated 6 weeks earlier. In A, the denervated muscle was electrically stimulated only for the final 12 days, while in B, the muscle was stimulated continually for the entire period. Neither muscle had a significant number of extra-junctional binding sites, but in both muscles the denervated end-plates bound only half the normal amount of toxin. Filled circles in A represent 5–10 end-plates each, in B all groups except the one with the smallest error bar contained 1–3 end-plates.





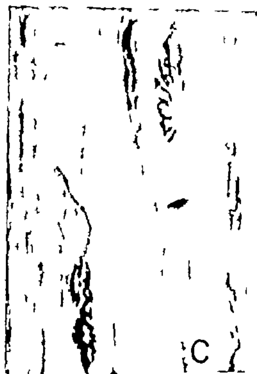




Plate 1 End-plates stained for ACh-esterase from soleus muscles th t had been incubated in ¹²⁵I-α-bungarotoxin. A. Denervated end-plate on a muscle fibre that had been denervated nd cross-i nervated f f months earlier Arrow marks filamentous strands of reaction product extending from the end-plate. B. Denervated end-plates on f bres that had been denervated and chronically timulated f al weeks. C As in B, but electrical stimulation was only for the final 1 days of the six week denervation period D A normal innervated end-plate from a unoperated soleus muscle. The marker 50 μm, and is f all pictures.

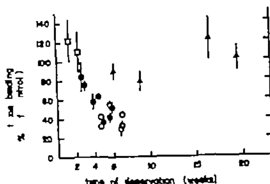


Fig. 4. Summary of results of toxin binding to denervated end-plates vs. time of denervation. Each point is the average toxin binding to all measured end-plates on one muscle expressed as a percentage of control binding to the normal end-plates on the contralateral muscle. □, denervated, unstimulated muscles; these values are the only ones corrected for extrajunctional binding (see Results). ●, denervated-stimulated muscles; ○, denervated muscles stimulated only for the final 1½–2 weeks to reduce extrajunctional binding; ▲, cross-innervated muscles. Error bars show ±1 S.E. of the mean, based on the variability of binding in different groups of end-plates from single muscles and taking into account the variable numbers of end-plates in each group. Denervation or denervation plus stimulation resulted in a slow loss of junctional binding sites over several weeks, whereas cross-innervation protected the sites at the old denervated end-plates for at least several months.

weeks for both groups. Denervated end-plates on cross-innervated fibres, on the other hand, retained their ability to bind toxin for at least several months.

Atrophy

Muscle fibres that were innervated by the foreign nerve appeared normal at the end of the experimental period whereas denervated and denervated-stimulated fibres were somewhat atrophic. We tested for a possible correlation between atrophy and loss of junctional binding sites by weighing the dissected, aldehyde-fixed muscles. These measurements are shown in Fig. 5: percent of normal (paired) muscle weight is plotted against the time after denervation. Several denervated, unstimulated muscles are included for comparison.

The two muscles denervated for 1.4 and 2.4 weeks weighed only half as much as their paired controls, yet end-plates on these muscles bound approximately normal amounts of toxin (Fig. 4). After four weeks of denervation the weights of muscles stimulated only for the final 1 1/2 weeks or for the entire period had also fallen to about half. It was therefore not possible to assess the reduction in number of junctional binding sites independently of muscle atrophy at these later times.

Discussion

Toxin binding to end-plates on denervated muscles

There was a considerable reduction in the number of toxin binding sites at end-plates on denervated muscle fibres with time. The electrical stimulation parameters employed in the present study apparently had little or no effect on the loss of toxin binding sites occurring at denervated end-plates. Muscles stimulated continuously from the time of denervation

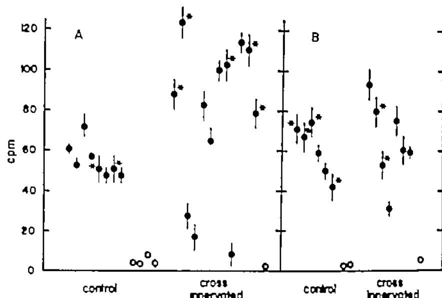


Fig. 3 Preservation of toxin binding to denervated end-plates on cross-innervated muscle fibres. The experimental muscle in A was denervated and cross-innervated (see Methods) 16.6 weeks earlier in B, 19 6 weeks earlier There was a greater than normal variation in the binding to individual end-plates, but on the average denervated end-plates in these muscles bound just as much toxin as normal innervated end plates did. Each point marked represents a single end-plate other groups contained 2 7 end-plates.

bars indicate the uncertainty in the measurement of radioactivity of each group of end-plates. The experimental muscle shown in Fig. 2 A was denervated for a total of 5 7 weeks and was stimulated for the last 12 days in order to eliminate extrajunctional binding, while in Fig. 2 B the muscle was stimulated continually from the time of denervation for a total of 6 weeks. Chronic stimulation provided no protection against the reduction in toxin binding in both cases the end-plate binding fell to about half of the control values.

The loss of binding sites from end-plates denervated for several weeks was in sharp contrast to results obtained for denervated end-plates on fibres innervated by another nerve but at a distant location. Two examples are presented in Fig. 3 The end-plates on one muscle, Fig. 3 A, had been denervated for 16 1 weeks and in Fig. 3 B for 19 6 weks. On the average the original ("old") end-plates in these muscles bound just as much toxin as normal end-plates did, despite the fact they had been denervated for over four months. Denervated end-plates on fibres cross-innervated for shorter times also bound normal amounts of toxin (see Fig. 4) When single end-plates were dissected out and assayed, there was considerable variation in the amount of toxin bound to each end-plate (points marked in Fig. 3). Some bound more toxin than normal while others bound almost none, even though all the fibres were cross-innervated (see Methods). There is a similar variability in the ACh sensitivity of denervated end-plates on cross-innervated muscle fibres (Frank *et al* 1975)

The combined results of toxin binding to all denervated end-plates are summarized in Fig. 4 Binding is expressed as a percentage of the average binding to normal soleus end-plates of the same rat. There is a slow reduction in the number of toxin binding sites on denervated end-plates both for fibres stimulated from the time of denervation and fibres stimulated only at the end of the denervation period. On the average toxin binding fell 60–70 % in four to

is also of interest that toxin binding was greatly reduced at denervated end-plates ome of the cross-innervated muscle fibres. This variability between individual end-plates l be related to the time at which cross-innervation of a particular fibre actually occurs. example, when a foreign nerve axon innervates a denervated muscle fibre at a new loca- it might preserve the current level of esterase activity and number of toxin binding sites e old end-plate. Alternatively it may be that over long periods of time innervation at a nt site is simply insufficient to prevent changes in the original end-plate and that different s undergo these changes at different rates.

able mechanisms of junctional receptor maintenance

re was a striking difference between the amount of toxin bound to denervated end- es in muscles with and without cross-innervation. One factor that might explain this erence is that denervated muscles underwent atrophy whether they were electrically ulated or not whereas cross-innervated fibres remained normal in size. Loss of toxin ding sites might occur *pari passu* with a general reduction in muscle size.

he two muscles denervated for only 1.4 and 2.4 weeks indicate that there is not a constant tionship between junctional receptors and muscle weight. Although the muscles had lost f their original weight the end-plates bound apparently normal amounts of toxin. These ilts, however are still compatible with the idea of a delayed loss of junctional receptors owing general muscle atrophy. The experiments with denervated-stimulated muscles not resolve this issue because electrical stimulation did not completely block muscle ophy. The stimulation parameters were sufficient to suppress extra-junctional toxin ding, but it is possible that more adequate stimulation might also preserve junctional ding sites.

An alternative possiblity is that the foreign nerve exerted some direct trophic effect er the original, denervated end-plates independent of its role in activating the muscle. uth *et al.* (1966) suggested a similar hypothesis to explain the preservation of ACh esterase tivity at denervated end-plates on cross-innervated muscles. Whatever the mechanism rns out to be, the persistence of junctional receptors at denervated end-plates on cross- nervated muscle fibres is remarkable.

pleasure to acknowledge the advice provided by T. Lømo, Drs J. K. S. Jansen and D. E. Van nce during all phases of this work. E. F. was supported by postdoctoral fellowship from the Muscular ystrophy Associations of America, Inc.

References

XILSON, J. and S. THESLEFF. A study of supersensitivity in denervated mammalian skeletal muscles. *J. Physiol.* (Lond.) 1959. *147*: 178–193.
ERG, D. K., R. B. KELLY, P. B. SARGENT, P. WILLIAMSON and Z. W. HALL. Binding of α-bungarotoxin to acetylcholine receptors in mammalian muscle. *Proc. nat. Acad. Sci.* (Wash.) 1972. *69*: 147–151.
IAMENO, L. and R. MILEDI. A study of foetal and newborn rat muscle fibres. *J. Physiol.* (Lond.) 1962. *161*: 393–408.
RANK, E., J. K. S. JANSEN, T. LØMO and R. WESTGAARD. The interaction between foreign and original motor nerves innervating the soleus muscle of rats. *J. Physiol.* (Lond.) 1975. In press.
REENWOOD, F. C., W. M. HUNTER and J. S. GLOVER. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* 1963. *89*: 114–123.

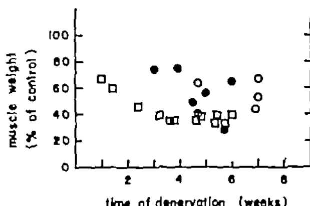


Fig. 5. Atrophy of denervated and denervated stimulated muscles vs. time. □ denervated not stimulated (some of this data was provided by T Lømo, personal communication), ○ denervated muscles stimulated only for the last 1½ 2 weeks, ● denervated-stimulated muscles. Atrophy is expressed as the weight of aldehyde-fixed denervated muscles compared to the contra lateral unoperated muscles. Stimulation provided some protection against atrophy but the protection was not complete.

had to be compared to muscles stimulated for the final 1 1/2 weeks nevertheless a pronounced preserving effect of continuous stimulation on the ACh receptors would probably have resulted in some discrepancy between the binding to the two types of end-plates. No such discrepancies were observed. A simple hypothesis that is consistent with the data is that toxin binding to denervated end plates falls steadily over several weeks whether the denervated muscle is stimulated or not

ACh esterase associated with denervated end-plates also declines with time. Guth *et al* (1964) reported a fall to 25% in two weeks for the rat's sternomastoid muscle and Hall (1973) found that seven days after denervation end-plate specific esterase had fallen to 21% of normal in the diaphragm. The early loss of esterase was somewhat faster than the decline of muscle weight (Guth *et al* 1964) Thus after denervation the loss of end-plate esterase begins before fibre atrophy and subsequent loss of toxin binding sites. After four to six weeks, however levels of esterase activity and numbers of binding sites are reduced to approximately the same extent (Guth *et al* 1964 Guth, Zalewski and Brown 1966).

Toxin binding to cross-innervated muscles

In contrast to the loss of toxin binding sites from end-plates on denervated muscles, most end plates on cross-innervated muscle fibres showed no diminution of toxin binding even after four months The distribution of these binding sites is not known microscopically. At normal end-plates, Hartzell and Fambrough (1972) have shown that toxin binding is highly restricted to the synapse itself as revealed by combined autoradiography and ACh esterase stains. In view of the faint filamentous appearance of the esterase stain at some old denervated end-plates on cross-innervated fibres, it would be interesting to know the microscopic distribution of toxin binding sites and to compare at single end plates the number of toxin sites with quantitative determinations of esterase activity

The maintenance of normal toxin binding characteristics of the denervated end-plate on cross-innervated muscles is reminiscent of the maintenance of old end-plate esterase activity on cross-innervated sternomastoid muscles of rats reported by Guth *et al* (1966). These authors found that after 8 weeks of denervation and cross innervation the esterase activity in the old end-plate region was half that of normal controls, whereas with denervation

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Differential Effects of Temperature on Activity of LDH from Seal and Sheep Skin

By

ARNOLDUS SCHYTTE BLIX, EDVARD B. MESSELT and HANS J. GRAV

Received 4 March 1975

Abstract

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homeothermic seal possesses heterothermic skin, while the skin of the sheep behaves as truly meothermic tissue. A new method for skin homogenization is described. The effect of temperature on catalytic behaviour of seal and sheep skin LDH has been investigated by fluorometric activity measurements, with results presented as Arrhenius plots. The seal skin enzyme had ten times higher activity at temperatures as compared to the sheep skin. The mechanisms responsible for this different behaviour discussed

cold ater the skin temperature of arctic seals approaches that of the aqueous environment (Irving and Hart 1957). While basking in the sun, the skin of such animals frequently quires temperatures exceeding 40°C (Øritsland 1970). This means that the homeothermic al possesses a heterothermic skin. Conversely sheep skin is protected by a thick woolly oat, and behaves like truly homeothermic tissue (.f Fig. 2).

In order to retain metabolic function in wide heterothermic range, the seal skin enzymes should possess catalytic activities quantitatively different from those of the homeothermic sheep skin. As a possible candidate for such adaptive change we therefore chose to nvestigate possible differences in temperature sensitivity of lactate dehydrogenase (E.C. .1.1.27) from the skin of these animals. This key glycolytic enzyme was selected because t is crucial to skin survival, both under anaerobic (seal during diving) as well as aerobic conditions

Methods

Subcutaneous and rectal temperatures of an adult female hooded seal (*Cystophora cristata*) were recorded by use of thermocouples connected to Speedomax multi-channel recorder. The studies were carried out aboard sealing vessel in the North Atlantic Ocean, off Jan Mayen. Ambient temperature was −5°C and the wind speed 13 m/s. The recordings were continued with the animal submerged (diving) in sea water of 1.5°C.

GUTH, L., R W ALBERS and W C. BROWN, Quantitative changes in cholinesterase activity of denervated muscle fibers and sole plates. *Exp Neurol* 1964 *10*, 236–250.

GUTH, L., A. A. ZALEWSKI and W C. BROWN, Quantitative changes in cholinesterase activity of denervated sole plates following implantation of nerve into muscle. *Exp Neurol* 1966. *16* 136–147

HALL, Z. W Multiple forms of acetylcholinesterase and their distribution in endplate and nonendplate regions of rat diaphragm muscle. *J Neurobiol.* 1973 *4* 343–361

HARTZELL, H. C. and D M FAMBROUGH, Acetylcholine receptors distribution and extrajunctional density in rat diaphragm after denervation correlated with acetylcholine sensitivity *J gen. Physiol* 197? *#* 248–262.

HOFFMAN, H A study of the factors influencing innervation of muscles by implanted nerves. *Aust J exp Biol. med Sci.* 1951 *29* 289–308

KARNOVSKY M. J The localization of cholinesterase activity in the rat cardiac muscle by electron microscopy *J Cell Biol.* 1964 *23* 217 232

LØMO, T and J ROSENTHAL, Control of ACh sensitivity by muscle activity in the rat. *J Physiol.* (Lond) 1972. *221* 493–513

MILEDI, R. and L. T POTTER, Acetylcholine receptors in muscle fibres. *Nature* (Lond.) 1971 *233* 599–603

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Methods

Subcutaneous and rectal temperatures of an adult female hooded seal (*Cystophora cristata*) were recorded by use of thermocouples connected to Speedomax multi-channel recorder. The studies were carried out aboard sealing vessel in the North Atlantic Ocean, off Jan Mayen. Ambient temperature was −5°C and the wind speed 13 m/s. The recordings were continued with the animal submerged (diving) in sea water of 1.5°C.

Recordings of subcutaneous temperatures were also gathered from domestic sheep exposed to air temperatures ranging from −20 to +40°C in a temperature controlled room.

Animals and samples

Ringed seals (*Pusa hispida*) and domestic sheep were used in this study. The seals were obtained from Bay, Spitzbergen, and the sheep from a farm outside Oslo, Norway. Skin samples were excised from hind flipper (leg) of the seals and from the middle back of the sheep, and frozen in liquid nitrogen within 10 min after sacrificing the animal. The samples were thereafter kept at −85°C.

Homogenization

The samples were cut in sections 10 μm thick on a Junge microtome mounted in a Linde cryostat at ... About 100 sections were assembled in a high speed centrifuge tube containing 1 ml 0.1 M Na_2... NaH_2PO_4 buffer pH 7.4. The tubes were incubated at room temperature for 10 min and centrifuged for 1 h at 50 000 × g in a Sorvall RC2 B centrifuge. The supernatants were used as enzyme source immediately after centrifugation.

Protein

Protein was determined according to Lowry *et al.* (1951). Electrophoresis was performed according to Dietz and Lubrano (1967), as modified by Dietz *et al.* (1970) and by Fritz *et al.* (1970).

Enzyme assay

Maximal LDH activity was determined by spectrophotometric assessment of rates of oxidation of NADH (Sigma) at different substrate (pyruvate, Sigma) concentrations. The measuring wavelength was 3... The incubation mixture contained 0.1 M sodium phosphate buffer pH 7.4, pyruvate in concentrations ranging from 30 μM to 10 mM, 4 mM NADH, and 0.1 ml of the supernatant, in a total volume of ...

Effect of temperature on LDH catalytic behaviour

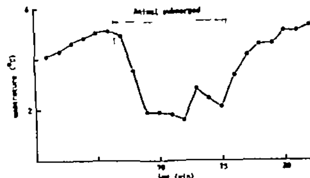
Arrhenius plots for seal- and sheep skin LDH were constructed from the specific activity values obtained at different temperatures. For this purpose LDH activity was measured fluorimetrically using a reaction mixture containing the following final concentrations of components: 5 μM NADH, 100 mM tris(hydroxymethyl)amino-HCl buffer, pH 7.2, and enzyme in 0.1 M sodium phosphate buffer. The pyruvate concentration was so adjusted that enzyme activity remained within 10% of maximal activity under all experimental conditions. Oxidation of NADH was followed with an Eppendorf photometer (Model M 1911) with fluorimetry attachment and recorder adapter for signal amplification. The reaction was started by the addition of pyruvate and internally standardized by adding 0.1 nmol NADH prior to termination of the reaction. The chamber holding the cuvette was thermocontrolled by the electronic device described by Näh... *et al.* (1974).

Results

While resting in air at −5°C, the hooded seal revealed subcutaneous temperatures of 13.2 ± 0.2 S.D. and 24.2 ± 1.0 S.D. °C respectively at two different locations (the first exposed to a wind speed of 13 m/s, the second leeward). Rectal temperature was 3... Upon diving, the animal displayed a drop in subcutaneous temperature. Fig. 1 illustrates the changes in subcutaneous temperature during a dive in ice water after a series of submersions.

Regardless of ambient temperature, the subcutaneous temperature of the sheep was constant at 38°C (Fig. 2). This value is close to the animal's deep body temperature.

The electrophoretic analysis of the isoenzyme pattern exposed twice as much M-subunit in the seal skin enzyme as in the sheep skin (Table I). The seal skin enzyme, furthermore, exhibited maximum activity at the higher substrate concentration (Table I), as would be expected from the isoenzyme patterns (Kaplan *et al.*, 1968).



1 Subcutaneous temperature hooded seal in response to ... in ice water after ex... re to ambient temperatures 5°C, following ... series of ... t submersions. Animal cold ... pted (arctic winter).

Fig. 3 illustrates the effect of temperature on total LDH catalytic activity within the range ...-42.9°C. *The Arrhenius plots reveal that specific enzyme activity* is higher in the seal skin ...zyme throughout this range. At low temperature (3.5°C), the sheep skin enzyme is 10 ...mes less active on a protein basis. This difference in catalytic behaviour becomes strikingly ...parent from the calculated activation energies, that of the sheep skin requiring higher ...ergy input. Moreover the Arrhenius plot of the seal skin enzyme is characterized by an ...rupt decrease in activation energy as the temperature is lowered beyond 20°C. No such ...viation from the behaviour predicted by the Arrhenius equation is observed with sheep ...in LDH.

Discussion

...veral studies (...g. Irving and Hart 1957 and Ray and Fay 1967) have indicated that the ...al skin has to tolerate temperatures below 20°C for prolonged periods in the course of ...rctic winter. Moreover during diving the blood flow through the vascular bed of the skin ... arrested, and energy production consequently has to proceed anaerobically at tempera-...ures close to 0°C (Fig. 1). The sheep skin, on the other hand, seems to be well circulated

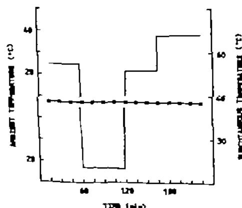


Fig. 2. Subcutaneous temperature of cold adapted sheep (●), in response to changing ambient temperature (—).

TABLE I Percentage distribution of LDH isoenzymes obtained electrophoretically from homogen seal and sheep skin. The table also includes the calculated M-subunit content and the sub (pyruvate) concentration of maximal catalytic activity of the enzyme.

	Percentage distribution of isoenzymes Isoenzyme number					Percent M subunit	Substrate concentr at maxim activity (
	1	2	3	4	5		
Seal	—	6	22	28	44	78	10^{-3}
Sheep	26	16	43	11	4	38	$3\ 10^{-4}$

and maintained at a temperature close to that of the central core, independent of the th environment (Fig. 2)

This functional difference between the two types of skin tissue is reflected in the ingly different catalytic behaviour of their lactate dehydrogenases, an important enzy the intermediary metabolism Thus, the enzyme from seal skin exhibited an activ times greater than that of sheep skin at low temperatures (Fig. 3)

As to the mechanisms which allow this different behaviour it is apparent from th rhenius plots that the sheep skin enzyme calls for much higher activation energies th seal skin enzyme throughout the biologically important temperature range (Fig. 3) break in the plot makes this difference particularly acute at low temperatures where th skin enzyme is characterized by an activation energy only half that of sheep skin enz

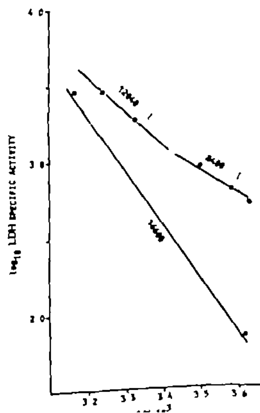


Fig. 3 Arrhenius plots for seal (●) and shee skin LDH showing the differential effect o perature on the catalytic behavior of the en LDH specific activity is given as µmol N oxidized min mg protein The figures each plot represent activation energy (cal calculated over a given temperature range break in the plot for seal skin LDH occurs at R nge of temperatures 3.5–42.9°C.

ch breaks in the Arrhenius plot have occasionally been shown to be a result of direct nce of temperature on protein structure (e.g. Massey 1953). Irrespective of explana- plots of this peculiar shape reflect catalytic properties of great benefit for enzymes d to function for prolonged periods at very low temperatures.

cording to Fritz (1967), the LDH 5 (M_4) from rabbit muscle increased its activity than 200% as the pH of the medium decreased from 7.8 to 6.4. The LDH from seal consists almost exclusively of M-subunits (Table I). It therefore seems likely that a lar increase in enzyme activity can take place in the seal skin in response to the low pH rred during diving. Massey (1953) has demonstrated that a similar effect on the enzyme rase is caused by changes in activation energy

he higher enzyme activity in the seal skin at low temperatures, as compared to the sheep , may also result from an enhanced enzyme-substrate affinity as reported for the hetero- mic flipper artery LDH of the common seal (Somero and Johansen 1970).

uperficially the observed isoenzyme pattern of the seal skin seems to agree with postu- d catalytic function under anaerobic conditions (Everse and Kaplan 1973). However lemonstrated by Berg and Blix (1973), different cell types of the same tissue may exhibit erent isoenzyme patterns. The skin is a tissue of extreme cellular heterogeneity and ilable data is insufficient to allow discrimination of differing metabolic activities in the ious cell types. It follows that it is not yet possible to decide whether the observed dif ntial effects of temperature on LDH activity (Fig. 3) is a direct consequence of the dif ent isoenzyme patterns of the two types of skin.

Whatever the mechanism, the skin is maintained in a functional state throughout the le temperature range (4–40°C) by the seal although, as demonstrated by Feltz and Fay 966), epidermal cells from great many species of Pinnipeds require temperatures above °C for proliferation. The survival of the seal skin during arctic winter therefore, has to pend on its unusual ability to endure prolonged (2–6 months) hypothermia of 4°C (Feltz d Fay 1966)

are grateful to Prof. O. Lindberg and Dr J. Vallin of the Wenner-Gren Institute, Kungl. Universitetet ockholm for generously providing the facilities and equipment necessary for fluorometric measurements. This investigation was supported by the Norwegian Council for Science and the Humanities, the Nor- egian Fishing Council and Norsk Polarinstitutt (Norwegian Polar Institute).

References

erg, T and A S Blix, Distribution of lactate dehydrogenase isoenzymes in rat liver cells. *Nature New Biol* (Lond.) 1973, 245 239–240.

Dietz, A A and T Lubrano, Separation and quantitation of lactate dehydrogenase isoenzymes by disc electrophoresis *Analyt Biochem.* 1967 20 246–257

Dietz, A A., T Lubrano and H. M. Rubinstein, Disc electrophoresis of lactate dehydrogenase isoen- zymes. Clin chim. *Acta* 1970. 27 225–232.

Everse, J and N. O. Kaplan, Lactate dehydrogenases Structure and function. *Advanc. Enzymol.* 1973. 37 61–133

Feltz, E T and F H F Thermal requirements in vitro of epidermal cells from seals. *Cryobiology* 1966 3. 261 264.

Fritz, P J Rabbit lactate dehydrogenase isozymes Effect of pH on activity *Science* 1967 *156*. 82–83.

Fritz, P J W J Morrison, E. L. White and E. S Vesell, Comparative study of methods for quantita- tion of lactate dehydrogenase isoenzymes. *Analy Biochem.* 1970. 36. 443–453.

IRVING, L. and J S. HART The metabolism and insulation of seals as bareskinned mammals in cold w
Canad J Zool 1957 *35* 497 511

KAPLAN, N O., J EVERSE and J ADMIRAAL, Significance of substrate inhibition of dehydrogenases. *A N Y Acad Sci* 1968, *151* 400–412.

LOWRY O H H J ROSEBROUGH, A. L. FARR and R. J RANDALL, Protein measurement with the F phenol reagent. *J biol Chem.* 1951 *193* 265–275.

MASSEY V Studies on fumarase: The effect of temperature. *Biochem. J* 1953, *53* 72 79

NÄSLUND P B. LILJESVAN and L. ABRAHAMSSON, High resolution analyzer of thermal denaturation *Analyt Biochem.* 1974 *57* 211 18

ØRITSLAND, N A., Energetic significance of absorption of solar radiation in polar homeotherms *Antarctic Ecology* (Ed. M. W Holdgate), *1* 464–470. Acad. Press. 1970.

RAY C. and F H FAY Influence of climate on the distribution of walruses. II Evidence from phys logical characteristics. *Zoologica* 1968, *53* 19–3.

SOMERO, G N and K. JOHANSEN Temperature effects on enzymes from homeothermic and heterother tissues of the harbor seal. *Comp Biochem. Physiol* 1970 *34* 131 136.

Acta physiol. scand. 1975. 95. 83–88

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Effect of Spinal Sympathetic Blockade upon Local Regulation of Blood Flow in Subcutaneous Tissue

By

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Abstract

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he influence of spinal sympathetic blockade upon local regulation of blood flow in subcutaneous adipose sue was investigated in six subjects. The effect of changes in orthostatic pressure on blood flow in subcutaneous tissue in the arm and distal forearm was measured before and after sympathetic blockade obtained by epidural anaesthesia in 4 subjects and by bilateral sympathectomy in 2 patients suffering from essential hyperhidrosis. Blood flow in subcutaneous tissue measured by ^{133}Xe washout technique decreased about 40 per cent when the limb was lowered, and remained constant during 30 cm elevation. This was und both before and after the blockade, though in one of the patients, the orthostatic decrease in blood ow was less pronounced 24 h after sympathectomy. Hence central sympathetic reflexes do not alter local thostatic changes of blood flow in subcutaneous tissue. These changes therefore are most likely due to cal mechanisms.

lood flow in subcutaneous adipose tissue of a limb remains almost constant when the region studied is displaced within approximately 20 cm above and 20 cm below heart level. utside these levels, blood flow decreases (Henriksen *et al.* 1973, Paaske and Henriksen 973). During elevation, perfusion pressure decreases as venous pressure remains constant. his indicates that blood flow is kept constant by a decrease in vascular resistance. When he region studied is positioned more than 20 cm below heart level, a decrease in blood low must be due to an increase in vascular resistance as arterial and venous pressure increases in parallel below heart level.

These changes in vascular resistance could be due to changes in sympathetic vasoconstrictor activity. Stimulation of sympathetic nerves induces vasoconstriction in subcutaneous adipose tissue of the dog (Öberg and Rosell 1967). The contribution of vessels in the skin o changes in resistance induced by postural changes is still matter of debate. Roddie and Sheperd (1956, 1957), Roddie *et al.* (1958), concluded that skin vessels do not contribute o the regulation of the total resistance during orthostatic pressure changes and changes in arterial baroreceptor activity (Roddie and Sheperd 1956, Roddie and Sheperd 1957. Roddie

et al 1958) This conclusion has been supported by others (Delius *et al* 1972, Hanley and Skinner 1971 Ngai *et al* 1969) However other investigators found that skin do contribute (Mosley 1969 Beiser *et al* 1970 Rowell *et al* 1973).

The present study deals with the possible role of sympathetic nerves in the ob changes in vascular resistance during local orthostatic pressure changes (Henriksen 1973 Paaske and Henriksen 1973)

Experimental procedure

Epidural anesthesia. The experiments were carried out on 4 subjects referred to hospital for hern to be performed in epidural anesthesia. The subjects were placed in the examination room 90 min the measurements were started. Room temperat re was about 21°C and remained constant investigation. Skin temperature was measured. The subjects were placed in a supine position supported at the crus and heel so that the areas under study could be placed at levels below or ab mid-axillary line.

^{133}Xenon was applied to subcutaneous tissue by means of the intracutaneous injection technique (1971). About 90 min after the injection ^{133}Xe is exclusively located in subcutaneous tissue. This tec was chosen in order to avoid trauma to the subcutaneous tissue. The areas under study were locate fibular head and at the lateral malleolus. Both legs were investigated.

A catheter was placed in the epidural space correspondi g to the third lumbar vertebra.

Blood flow in subcutaneous tissue was measured consecutively with the legs placed horizontally in such a way that the proximal and distal depots were lowered 30 and 40 cm below the mid-axilla respectively and then returned to the horizontal position. Hereafter the legs were elevated so that depots were raised 30 cm above the mid-axillary line. Finally the blood flow was measured with placed horizontally Each measurement lasted about 8 min.

Marcain 0.5%, without adrenaline, was injected into the epidural space through the cathet volume was 1.5 ml per segment. The blockade was extended to involve the 6th to 8th thoracic se Increase in skin temperature measured on the big toes (Roe *et al.* 1973), disappearance of the symp galvanic reflex (Daos *et al* 1963), and abolished sweat production, tested by the ninhydrin test (I *et al* 1960), were taken as evidence of an effective sympathetic blockade. After termination of vestigation the sweat test was negative. In order to avoid a decrease in arterial blood pressure, isotonic saline were infused before the blockade was started. Arterial blood pressure was measur remained constant throughout the investigation.

After the sympathetic blockade had been achieved, measurements of blood flow in the same are repeated as described above. The plasma concentration of adrenaline and nor-adrenaline was me before and after the sympathetic blockade. Samples of 20 ml blood were taken from a superficial determination of these concentrations according to Valori *et al* (1973), using an Aminco-Bowman fluorometer (Type D 223-62155).

Bilateral sympathect my

Two patients sufferi g from manual hyperhidrosis were examined before and 24 h after partial b sympathectomy was performed. Blood flow in subcutaneous tissue in the distal part of the forea measured with the investigated area placed t reference level (jugular notch) and lowered 30 and below the jugular notch.

A section of the sympathetic chai with one ganglion above and one below the third rib was re After the operation sweat test was negative.

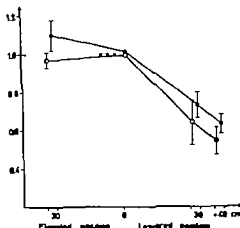
Calculations

Blood flow was measured by means of the ^{133}Xe washout technique (Sejrsen 1971).

The perfusion coefficient, f was calculated from the Kety equ tion

$$f = k \cdot \lambda \cdot 100 (\mathrm{ml}/100\ \mathrm{g\ min})$$

where k denotes the rate constant of the washout functio (min) and λ the adipose tissue to blood tion coefficient (ml/g) (Kety 1951). I the calculations, a λ-value of 10 was used (Sejrsen 1971). The



1. Relative change in mean blood flow ($f_{(test)}/f_{(ref)}$) in subcutaneous tissue during positional changes of limb before and after sympathetic blockade. ○ – before, ● – after. The ... denote number of experiments. The vertical lines denote one standard error of the mean.

...t rate constant, k, was computed from the regression line of the logarithmically transformed count ... (corrected for background activity) by means of the method of least squares.

...atistics

...ean perfusion coefficient obtained at reference level ($f_{(ref)}$) was compared to mean perfusion coefficient ...btained at test level ($f_{(test)}$) by means of Students' t-test for paired samples. $f_{(test)}/f_{(ref)}$ and the concentration in plasma of adrenaline and nor-adrenaline, measured before and after sympathetic blockade, were compared by the randomization test for paired samples, respectively. $f_{(ref)}$ measured before and after sympathetic blockade was compared by the randomization test for independent samples. As level of significance was chosen 0.05.

Results

Epidural anesthesia. The results are shown in Fig. 1. In all cases an increase in skin temperature of about 3–5°C was found after epidural anesthesia. Blood flow measured with the leg placed horizontally before and after sympathetic blockade is shown graphically in Fig. 2. Mean $f_{(ref)}$ increased significantly from 6.0 ml/100 g min (range 1.3–10.2) to 8.4 ml/100 g min (range 2.9–16.9) after the blockade ($p < 0.005$). The average concentration in plasma of adrenaline increased from 53 ng/l plasma (range 0–257) before to 256 ng/l plasma (range 0–420) after sympathetic blockade. Normal range: 0–337 ng/l plasma, $p < 0.05$

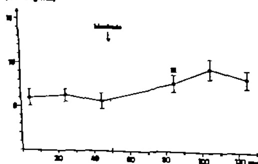


Fig. 2. Average blood flow in subcutaneous tissue measured with the leg placed horizontally before and after epidural anesthesia. The figures denote the number of experiments. The vertical lines denote one standard error of the mean.

et al 1958). This conclusion has been supported by others (Delius *et al* 1972, Hanley Sadi and Skinner 1971 Ngai *et al.* 1969) However other investigators found that skin vessels do contribute (Mosley 1969 Beiser *et al* 1970 Rowell *et al* 1973).

The present study deals with the possible role of sympathetic nerves in the observed changes in vascular resistance during local orthostatic pressure changes (Henriksen *et al* 1973 Paaske and Henriksen 1973)

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^{133}Xenon was applied to subcutaneous tissue by means of the intracutaneous injection technique (Sejrsen 1971). About 90 min after the injection ^{133}Xe is exclusively located in subcutaneous tissue. This technique was chosen in order to avoid trauma to the subcutaneous tissue. The areas under study were located at the fibular head and at the lateral malleolus. Both legs were investigated.

A catheter was placed in the epidural space corresponding to the third lumbar vertebra.

Blood flow in subcutaneous tissue was measured consecutively with the legs placed horizontally lower in such a way that the proximal and distal depots were lowered 30 and 40 cm below the mid-axillary line respectively and then returned to the horizontal position. Hereafter the legs were elevated so that the two depots were raised 30 cm above the mid-axillary line. Finally the blood flow was measured with the legs placed horizontally Each measurement lasted about 8 min.

Marcain, 0.5 %, without adrenaline, was injected into the epidural space through the catheter. The volume was 1.5 ml per segment. The blockade was extended to involve the 6th to 8th thoracic segment. Increase in skin temperature measured on the big toes (Roe *et al.* 1973), disappearance of the sympathetic galvanic reflex (Daos *et al.* 1963), and abolished sweat production, tested by the ninhydrin test (Dhuner *et al.* 1960), were taken as evidence of an effective sympathetic blockade. After termination of the investigation the "sweat test" was negative. In order to avoid a decrease in arterial blood pressure, 500 ml isotonic saline were infused before the blockade was started. Arterial blood pressure was measured and remained constant throughout the investigation.

After the sympathetic blockade had been achieved, measurements of blood flow in the same areas were repeated as described above. The plasma concentration of adrenaline and nor-adrenaline was measured before and after the sympathetic blockade. Samples of 20 ml blood were taken from a superficial vein for determination of these concentrations according to Valori *et al* (1973), using an Aminco-Bowman spectrofluorometer (Type D 223-62155).

Bilateral sympathectomy

Two patients suffering from manual hyperhidrosis were examined before and 24 h after a partial bilateral sympathectomy was performed. Blood flow in subcutaneous tissue in the distal part of the forearm was measured with the investigated area placed at reference level (jugular notch) and lowered 30 and 40 cm below the jugular notch.

A section of the sympathetic chain with one ganglion above and one below the third rib was removed. After the operation, "sweat test" was negative.

Calculations

Blood flow was measured by means of the ^{133}Xe washout technique (Sejrsen 1971).

The perfusion coefficient, f was calculated from the Kety equation

$$f = k \cdot \lambda \cdot 100 (\text{ml}/100\ \text{g min})$$

where k denotes the rate constant of the washout function (min^{-1}) and λ the adipose tissue to blood partition coefficient (ml/g) (Kety 1951). In the calculations, λ value of 10 was used (Sejrsen 1971). The ad

on of vascular smooth muscles is due to a local mechanism. When the leg is elevated, o-arteriolar reflex mechanism is not likely because venous pressure remains constant. fore, the autoregulation of blood flow demonstrated during elevation is probably due intrinsic mechanism. A myogenic response of vascular smooth muscles to changes mural pressure, "myogenic theory" (Bayliss, 1902) and/or liberation by the tissue sodilating metabolites, "metabolic theory" (Anrep 1912, Hilton 1971) could be re- sible for this intrinsic mechanism. The vasoconstrictor response to increase in trans- l pressure might be due to a local myogenic mechanism or perhaps a local reflex anism.

e local vasoconstrictor response might be connected with regulation of capillary pres- Mellander *et al.* (1964) found that capillary filtration coefficient decreased in the feet mans when they were placed in a dependent position. This finding indicates that the constrictor response elicited when transmural pressure increases will counteract an in- e in capillary pressure and hence an increase in capillary filtration rate (edema protec factor)

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References

ep G On local vascular reactions and their interpretation. *J Physiol.* (Lond.) 1921 45 318–327

iss, W M., On the local reactions of the arterial wall to changes of internal pressure. *J Physiol.* ond) 1902. *28* 220–231

enfeld, P and I. Greenberg, Effect of Changes in Limb Posture on Peripheral Blood Flow *Circulat.* es. 1953, *1* 478–482.

n, G D R. Zelis, S E. Epstein, D. T Mason and E. Braunwald, The Role of Skin and Muscle sistance Vessels in Reflexes Mediated by the Baroreceptor System. *J clin. Invest.* 1970 *49* 225–231

ton, A. C. and E. Rosenberg, Effects of Raised Venous Pressure in the Circulation of the Isolated fused Rabbit Ear *Amer J Physiol.* 1956. *185* 465–470.

n, F G and R W Virtue, Sympathetic Block Persistence After Spinal or Epidural Analgesia. *J mer med Ass* 1963, *183*, 285–287

on, W K.-E. Hagbarth, A Hongell and B. G Wallin, Manoeuvres Affecting Sympathetic Out ow in Human Skin Nerves. *Acta physiol. scand* 1972. *84* 177–186.

uds, K G S Engman and A Wilhelm, Ninhydrin Test—An Objective Method for Testing Local anaesthetic Drugs *Acta anaesth. scand* 1960, *4* 189–198.

kow B Intravascular pressure as factor regulating the tone of the small vessels. *Acta physiol. scand.* 949 *17* 289–310.

ell, P and A C Burton, Local Postural Vasomotor Reflexes Arising from the Limb Veins. *Cir- ulat Res* 1953 *1* 27–39

ey F J and J B Scott, Effects of Flow Rate, Venous Pressure, Metabolites, and Oxygen upon Resistance to Blood Flow Through the Dog Forelimb. *Circulat. Res* 1964. *XIV XV Suppl. I* 49–59

ley H G R G Sachs and N. S. Skinner Jr., Reflex responsiveness of vascular bed of dog sub- cutaneous adipose tissue. *Amer J Physiol.* 1971 *220* 993–999.

nsen, O S. L Nielsen and W Paaske, Autoregulation of Blood Flow in Human Adipose Tissue. *Acta physiol scand* 1973 *89* 533–537

nson, P C and K. M. Hanson, Effect of arterial pressure on arterial and venous resistance of in- testine *J appl Physiol.* 1962. *17* 503–508.

nson, P C Autoregulatory responses of cat mesenteric arterioles measured in vivo. *Circulat Res.* 1968 *22* 199–212.

ety S. S. Theory and applications of the exchange of inert gas at the lungs and tissues. *Pharmacol. Rev.* 1951. *3* 1–41

The average concentration in plasma of nor-adrenaline was 280 ng/l plasma (rang 492) before and 279 ng/l (range 46–424) after sympathetic blockade. Normal range. ng/l p > 0 8

When the areas under study were lowered 30 and 40 cm below mid-axillary line, flow in subcutaneous tissue decreased to 73 and 57 per cent, respectively before (p < and to 57 and 63 per cent after the blockade (p < 0.001). There was no significant diff between this vasoconstrictor response to increase in transmural pressure obtained b and after the blockade (p > 0 8) During 30 cm of elevation, blood flow remained co before and after the blockade (p > 0.5 and p > 0.2 respectively). The difference in re obtained before and after the blockade was insignificant (p > 0 1)

Bilateral sympathectomy 24 h after the operation, blood flow at reference level h creased about 100 per cent. When the area under study was lowered, blood flow dec to 36 and 59 per cent (p < 0 005) in patient No 1 and 2, respectively before and to 8 59 per cent (p < 0 01) respectively after the operation

Discussion

When the leg is elevated perfusion pressure declines as venous pressure remains co The finding of a constant blood flow under these circumstances indicates a decre vascular resistance.

When the leg is lowered, perfusion pressure remains constant, but transmural pr is increased A finding of a constant blood flow in this case indicates that vascular resi remains constant, and consequently that the smooth muscles in the walls of the vessel increased their force of contraction just opposing the tendency of passive distension vessels due to the increase in transmural pressure. A decrease in blood flow indica increase in vascular resistance. Such changes in vascular smooth muscle activity m due to

1) *Reflex changes* in sympathetic vasoconstrictor activity due to changes in blood of the central veins and the heart (low pressure baroreceptors) (Roddie *et al* 1958, *et al* 1972, Rowell *et al* 1973)

2) *Spinal reflex mechanisms* where *e.g* distension of the veins in the extremity might arteriolar constriction.

3) *Local nervous mechanisms* (Haddy and Scott 1964 Gaskell and Burton 1953).

4) *Intrinsic vascular adjustments* to changes in transmural pressure (Bayliss 1902, F 1949 Johnson and Hanson 1962, Johnson 1968)

Blood flow in subcutaneous adipose tissue increased during sympathetic blockade indicates that sympathetic discharge contributes to the maintenance of the tonus vessels. The magnitude may be underestimated, because the concentration of adre in plasma increased.

Central sympathetic blockade did not alter the effect of changes in local ortho pressure on blood flow in subcutaneous tissue. These findings are in agreement with consfield (1953) and Gaskell and Burton (1953) When the limb was lowered blood decreased significantly 24 h after sympathectomy which indicates that the changes in

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ffinity of Noradrenaline and Dopamine for Neural α Receptors ediating Negative Feedback Control of Noradrenaline Secretion in Human Vasoconstrictor Nerves

By

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Abstract

e, L. and J. BRUNDIN, *Affinity of noradrenaline and dopamine for neural α-receptors ediating negative feedback control of noradrenaline secretion in human vasoconstrictor rves.* Acta physiol. scand. 1975. 95. 89–94

d superfused field stimulated biopsy specimens of human peripheral arteries and veins, preincubated H-(−)-noradrenaline (NA) to label the neural stores of NA, were used to study the potency of dop- (DA) and of NA as triggers of α-adrenoceptor mediated negative feedback control of sympathetic mitter secretion, evoked by stimulation with trains of 300 shocks at 1 Hz. In this preparation s found to be only slightly less potent than NA in depressing both the secretion of ³H-NA, and the tile response, evoked by nerve stimulation. DA depressed the contraction evoked by exogenous well, but to a very much smaller extent. On the other hand, DA was a very weak agonist on the ptors of the smooth muscle; nearly 1 000 times higher concentrations of DA were required to mimic ctions evoked by exogenous NA. The results show that the neural α-receptor function involved in l of NA secretion differs considerably from the α-receptors of e.g. smooth muscle, with respect to ivity to DA. It seems possible that the observed depressing effect of DA on NA secretion may be of acological and clinical interest. It may at least in part explain the vasodilating effect of DA infusions n.

e is by now considerable evidence that the secretion of noradrenaline (NA) from sym- etic nerves, in many tissues and species, is subject to local negative feedback control, iated by neural adrenoceptors (Farnebo and Hamberger 1971, Kirpekar and Puig , Enero et al. 1972, Starke 1972), capable of sensing the perineuronal concentration of , and of mediating restriction of further secretion of sympathetic transmitter. This pre aptic receptor function has been reported to be excited by α-agonists and blocked by α- agonists, and thus to be more closely similar to α- than to β-receptors of e.g. smooth scle. Recent observations in isolated guinea-pig vas deferens indicate that the neural eceptors in this tissue and species are more sensitive to adrenaline than to NA, while they highly insensitive to dopamine (DA) (Stjärne 1975). There may exist tissue and/or species ferences in this respect, DA has been reported to be about as potent as NA as inhibitor

MELLANDER, S., B. ÖBERG and H ODELRAM, Vascular adjustments to increased transmural pressure cat and man with special reference to shifts in capillary fluid transfer *Acta physiol. scand.* 1964 34–48.

MORLEY J G A Reduction in Some Vasodilator Responses in Free-standing Man. *Cardiovasc. Res.* 1969 3 14–21

NGAI S. H S. ROSELL and L. WALLENBERG, Nervous Regulation of Blood Flow in the Subcutaneous Adipose Tissue of Dogs. *Acta physiol. scand.* 1966. 68 397–403

NIELSEN, S. L. Adipose tissue blood flow determined by the washout of locally injected ^{133}Xenon. *Scand. J clin. Lab Invest* 1972. 29 31 36.

ÖBERG B. and S. ROSELL, Sympathetic Control of Consecutive Vascular Sections in Canine Subcutaneous Adipose Tissue. *Acta physiol. scand.* 1967 71 47 56.

PAASKE, W P and O HENRIKSEN, Influence of orthostatic blood pressure changes on blood flow in subcutaneous tissue of the crus. *Acta physiol. scand.* 1973. *Suppl* 396. 145.

RODDIE, J C. and J T SHEPHERD, The Reflex Nervous Control of Human Skeletal Blood Vessels. *Clin. Sci.* 1956. 15 433–440

RODDIE, J C., J T SHEPHERD and R. F WHELAN Reflex Changes in Human Skeletal Muscle Blood Flow Associated with Intrathoracic Pressure Changes. *Circulat R s.* 1958. VI 232 238.

ROE, F C. and F L. COHN, Sympathetic Blockade During Spinal Anaesthesia. *Surg Gynec Obstet.* 1973 136 265–268.

ROSENBERG, E., Local Character of the Veno-Vasomotor Reflex. *Amer J Physiol.* 1956. 185 472–475.

ROWELL, L. B., C. R. WYSS and G L. BRENGELMANN, Sustained human skin and muscle vasoconstriction with reduced baroreceptor activity *J appl Physiol.* 1973 5 639–643

SEJRSEN, P *Measurement of cutaneous blood flow by freely diffusible radioactive isotopes.* Thesis, Copenhagen 1971 Danish Medical Bulletin 1971 Suppl. 18.

VALORI, C., C. A. BRUNORI, V RENZINI and L. CARZA, Improved procedure for formation of epinephrine and nor-epinephrine fluorophors by the trihydroxyindole reaction. *Analyt Biochem.* 1970. 33 158.

ZOLLER, R. P A. L. MARK, F M ABBOUD P G SCHMID and D D HEISTAD The Role of Low Pressure Baroreceptors in Reflex Vasoconstrictor Responses in Man. *J clin. Invest* 1972. 51 2967–2972.

a prior to the next stimulation, the infusion was stopped 3 min after the end of the stimulation period. ne presence of 0.6 μM desipramine and 10 μM normetanephrine, the infused catecholamines did not the resting efflux of ³H. Each catecholamine infusion period was terminated by emptying the chamber washing the preparation twice, before superfusion was continued. When the contraction in response logenous catecholamines was determined, these were added to the mixing chamber at 6 min intervals, figures given under Results represent their final concentration. When the contraction reached its peak, pen of the Grass polygraph was lifted and the chamber washed twice before the next stimulation. statistical evaluation of the results was by Student' t-test. The figures given in the paper represent means E.

Results

chanical effect of nerve stimulation

mulation with 300 shocks at 1 Hz frequently slightly reduced the longitudinal tension of artery but caused in most preparations a distinct rise in longitudinal tension of the vein. wever the rise in tension was only small, and maximum amplification on the Grass polyph had to be used to visualize it at this sensitivity level the baseline of the polygraph was longer horizontal, but drifted gradually upwards. Moreover the preparation showed nsiderable fatigue on repeated stimulation the contractile responses declined fairly pidly (Fig. 1)

l NA secretion evoked by nerve stimulation

n stimulation with 300 shocks at 1 Hz, during the initial control period, Δt per shock had n average value of (4.50 ± 0.27) 10⁻⁴ (n = 22). Due to certain variability in the control lue of Δt, from one experiment to the other the relative rather than the absolute values ere used for calculation of the observed pharmacological effects. The figures for Δt in ach experiment were normalized in relation to the average obtained during the first two ontrol stimulations in that same experiment, which was given the value of 1 relative unit.

Effect of DA and NA on Δt

n 5 expts. DA was infused before NA (Fig. 1), and in 7 the order was reversed. DA 0.2 1 μM caused dose-dependent and reversible depression of Δt, to 69.7 ± 4.0% and 48.7 ± 4.2% of the control level, respectively NA was somewhat more potent in this respect, at 0.2 and 1 μM concentration it depressed Δt to 64.7 ± 3.6% and 36.1 ± 3.6% of the controls. The difference in potency was not significant (0.05 > p 0.01). The average IC_{50} (the concentration required to depress Δt to one half) for DA was 0.90 μM while that for NA was 0.45 μM.

Effects of DA and NA on mechanical response

At 0.2 1 μM concentration DA did not alter the longitudinal tension of the preparations. However it markedly dose-dependently and reversibly depressed the contractions evoked by nerve stimulation (Fig. 1). NA enhanced the resting longitudinal tension at 0.2 μM only moderately but at 1 μM very markedly (Fig. 1). At 0.2 μM NA the superimposed contraction evoked by nerve stimulation was usually moderately depressed, and at 1 μM NA strongly so (Fig. 1). I many cases the restitution of the contractile response after washing was very incomplete (Fig. 1).

of sympathetic neurotransmitter secretion, in cat spleen and nictitating membrane (Lag 1973).

In a recent study of isolated superfused field stimulated biopsy specimens of human blo vessels, it was found that vasoconstrictor nerves in man also seem to possess an α-adre ceptor function, capable of triggering negative feedback control of the secretion of N (Stjärne and Gripe 1973). The present paper reports results from further studies of th preparation, with the aim to compare the potency of DA with that of NA, as trigger these, presumably neural, adrenoceptors.

Material and Methods

The biopsy specimens were taken from 9 normotensive female subjects, aged 29 to 66 years, 8 of the were undergoing gynecological or obstetric surgery (3 cases of Caesarian section) and 1 as operat for cholelithiasis. Surgery was performed under various forms of routine general anaesthesia, in the cases with halothane/oxygen/nitrous oxide/celocurine. Premedication always included atropine 0.5 m in addition to promethazine 25 mg and pethidine 5 to 75 mg s.c. After Caesarian section was pe formed, oxytocin (Syntocinon®) 2 IU and sometimes Methergine (Sandoz) 0.2 mg were administered b injection into the myometrium.

The biopsy specimens consisted of 5–6 cm pieces of arteries and veins from the omentum, of a calib of about 1 1.5 mm, excised between ligatures. The tissue was immediately transferred to Tyrode solution (NaCl 0.8 %, KCl 0.02 %, $CaCl_2$ 0.02 %, $MgCl_2$ 0.01 %, N HCO 0.1 %, NaH_2PO 0.005 %, glucose 0.1%, sodium ascorbate 20 μg/ml and atropine sulphate 1 μg/ml), prebubbled with ca bogen gas (6.5 carbo dioxide in oxygen), for transportation to the laboratory. During the subsequent dissection to remov inert tissue and to separate artery f om vein care was taken to avoid damage to the perivascular plex of sympathetic vasoconstrictor nerves.

In order to label the neural t res of NA, the preparation was then incubated with 10 μCi (2.4 μ ^3H-(−)-NA (New England Nuclear Corp.) per ml Tyrode solution, at 35°C for 30 min. After 10 ch for one min each the preparation was mounted in a cylindrical chamber 6 mm in diameter and superfus with Tyrode solution, aerated with carbogen gas (final pH 7.4). The temperature in the chamber maintained at 31°C and the flow rate was 2.5 ml ml. The superfusate was brought into test tubes in fraction collector collection periods were 4 min. The longitudinal tension in the preparation as measur with Grass f rce displacement transducer nd recorded on a Grass Polygraph the resting tension w adjusted to 0.5 g.

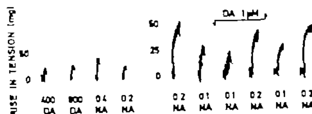
The ^3H level of one ml aliquots of the superfusate, nd of the tissue (t the end of the experime extracted with 0.4 M perchloric acid and then diluted ten-fold with distilled water was determined b counting in 10 ml Instagel (Packard Instr.) in an Intertechnique ABAC SL 40 Liquid scintillation spectr meter Quenching was monitored by nternal tandards the variation between samples in this respect wa negligible and was thus not corrected fo

The resting efflux f ^3H declined progressi ely throughout the experiments. The rise in H efflux evok by electrical field stimulation (t 90 V on a Grass S 44 timulator the preparat on was mounted betwe two parallel platinum wire electrodes, 6 mm apart and 40 mm in uninsulated length) with biphasic pulse 1.5 ms in duration, was completely blocked w th tetrodotoxin 3 μM and was thus clearly neural i origi Since practically ll of the H remaining in the tissue after washing was intact H NA, and since 0.6 μM desipramine and 10 μM normetanephrine were added to prevent rebinding of NA (I ersen 1971), the evok fractional rise (total efflux f $^3H(\Delta t)$

$$\Delta t = \frac{\text{Evoked rise in efflux of } H}{\text{Calculated tot l } ^3H \text{ in tissue t beginning of stimulation}}$$

was used t measure the secretion of H NA from the nerves. Ner stimulation w th trains of 300 shock at 1 Hz were pplied at 16 (or during washes 32) min intervals. Δt resulting from each stimulus train w calculated from the rise in ^3H efflux during three 4-min collection periods, starting from the beginning of the stimulation.

Exogenous catecholamines were dissolved n HCl (f nal concentration 0.4 μM), and infused startin



g 2. *Left panel* Typical record wing rise in longitudinal ten- of human omental vein, ed by addition of DA and of to the medium. The figures resent final μM concentrations. *ht panel*: Effect of DA 1 μM rise in longitudinal tension of man omental vein, caused by genous NA

ere is considerable reason to regard this as evidence for true receptor-mediated depression the secretion of neurotransmitter and not as an artefact, due to *e.g.* uptake and prefer tial secretion of exogenous unlabelled amine (*cf.* Stjärne 1975). Interestingly the observed C_{50} for NA on At in human vasoconstrictor nerves, 0.45 μM, was about the same as that und in guinea-pig vas deferens, under the same experimental conditions (Stjärne 1975). his is in contrast to the reported IC_{50} values for NA in *e.g.* rabbit heart (Starke 1972) or t spleen (Langer 1973) of about 0.06 μM. It is not possible at present to decide whether is discrepancy is due to methodology or to true tissue and/or species differences. How- ver the present results clearly indicate that the sensitivity level of the α-receptors of uman vasoconstrictor nerves is far too low to be affected by even the highest concentra- ions of NA which can occur in peripheral arterial plasma, under physiological conditions. On the other hand there can be little doubt that perineuronal concentrations of NA high nough to trigger α-adrenoceptor mediated negative feedback control of NA secretion do ccur in the immediate vicinity of actively secreting adrenergic nerve terminals, and partic ılarly in tissues with narrow synaptic clefts (Stjärne 1973 b, 1975, Stjärne and Gripe 1973).

The present results show that DA, while being a very weak agonist on the α-receptors of he smooth muscle of the preparation, was nearly as potent as NA as agonist on the neural α-receptors. This is in agreement with observations in cat spleen or nictitating membrane Langer 1973), but in distinct contrast to observations made in guinea-pig vas deferens, under identical experimental conditions, in this tissue DA was completely without effect on ³H NA secretion (Stjärne 1975). Thus there can be no doubt that there do exist true tissue and/or species differences with respect to the selectivity for catecholamines of the neural α-adrenoceptors mediating feedback control of NA secretion.

The contractile response to nerve stimulation, manifested as a rise in longitudinal tension of the veins, was difficult to demonstrate satisfactorily due to its smallness and rapid de- cline, on repeated stimulation. DA even at 1 μM reduced contraction in response to exo- genous NA only slightly Thus the observed marked reduction of the contractions in re- sponse to nerve stimulation, induced by exogenous DA and NA, strongly suggests that the simultaneous depression of ³H NA secretion was indeed a reflection of depression of secre- tion of total, endogenous sympathetic transmitter

It is not possible at present to judge whether the observed inhibitory effect of DA on the secretion of NA from sympathetic nerves in man is of physiological importance. However it may well be of clinical interest, it could explain at least in part, the observed vasodilator effects of intravenous administration of DA, in man (Horowitz *et al.* 1963 McDonald *et al.*

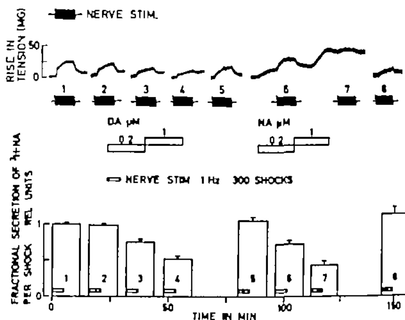


Fig. 1 *Upper panel:* Typical record showing effects of DA (dopamine) and of NA (noradrenaline) on rise in longitudinal tension of isolated superfused field stimulated human omental vein, induced by trains of 300 nerve shocks at 1 Hz. The figures 1–8 refer to stimulation periods in lower panel.

Lower panel: Fractional secretion of ³H NA per shock, in relative units (see Text under Results) nerve stimulation with 300 shocks at 1 Hz. Means ± S.E. (5 expts. in which DA was infused before N

Relative potency of DA and NA on contractile response

DA was very much weaker agonist than NA in evoking contraction. 400–800 μM conc trations of DA were required to mimick the contractions induced by 0.2–0.4 μM NA (Fig

Effect of DA on contractions evoked by exogenous NA

At 0.2 μM concentration DA was without consistent effect on the contractions evoked exogenous NA at 1 μM DA the responses to NA were very slightly depressed (Fig. 2).

Discussion

The aim of the present work was to compare the inhibitory effect of NA with that of D on the secretion of ³H NA from human vasoconstrictor nerves, evoked by trains of nerve shocks at 1 Hz. The low frequency was chosen because it has been found that efficiency of the receptor-mediated feed-back control of ³H NA secretions in other p parations is inversely related to nerve stimulation frequency (Stjärne 1973 a). The obser average control value for Δt per shock was in good agreement with that previously repor (Stjärne and Gripe 1973).

Exogenous (−)-NA dose-dependently and reversibly depressed Δt in agreement w previous observations in the same preparation (Stjärne and Gripe 1973). As pointed out a study of the control of ³H NA secretion from guinea-pig vas deferens (Stjärne 197

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The Pulmonary Vasoconstrictor Response to Hypoxia. Effects of Inhibitors of Prostaglandin Biosynthesis

By

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Abstract

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… purpose of the present work was to determine whether prostaglandins (PGs) synthetized in the …ediate the vasoconstrictor response to acute alveolar hypoxia. Isolated and ventilated lungs of rats …rfused at 37°C with homologous blood at constant-volume, pulsatile inflow and pressor responses …n periods of standardized ventilation hypoxia recorded. Indomethacin, sodium meclofenamate and …licylic acid (all 100 μg/ml), which are potent inhibitors of PG biosynthesis, did not reduce the … vasoconstrictor response. Sometimes they even enhanced this response. W conclude that PGs … mediate the hypoxia-induced vasoconstriction W suggest that vasodilatory PGs might act to re… …d modify pulmonary arterial hypertension due to hypoxia.

…mechanism whereby acute alveolar hypoxia elicits pulmonary vasoconstriction still re…s to be properly clarified. Since von Euler and Liljestrand (1946) demonstrated in cats …inhalation of subnormal concentrations of oxygen produced an increase in pulmonary …ial pressure, a number of vasoactive substances have been suggested—and discarded, …ssible humoral transmitters of this response. During the last few years several investi…rs in this field have focused their interest on histamine and other vasoactive agents …ained within mast cells situated near small muscular arteries in the lung (Hauge 1968 b, …ge and Staub 1969 Susmano and Carleton 1971 Haas and Bergofsky 1972, Kay *et al* …). The role of histamine has, however been disputed by some workers (Silove and …cha 1973).

…n the present work we have focused our attention on another group of substances, name…he prostaglandins (PGs). The lungs have a large capacity for synthesis and liberation of … secondary to various mechanical and chemical stimuli (Piper and Vane 1971 Piper …4). Of the various PGs synthetized within the lung, $PGF_{2\alpha}$ is a potent constrictor in the …monary circulation (Änggård and Bergström 1963). Furthermore, PGs have been sug…ted to control ventilation/perfusion ratios in the lung (Liljestrand 1967 Piper and Vane …1 Said 1973). PGs are released from lungs by ventilation (Berry Edmonds and Wyllie …71), and PG-like activity of the venous effluent from lungs has been reported to increase

1964 Rosenblum *et al* 1972) This possibility is supported by the recent evidence t induces vasodilatation in dogs, by blocking neurogenic vasoconstriction (Wille Bogaert 1975)

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References

ENERO, M A., S. Z. LANGER, R P ROTHLIN and F J E. STEFANO, Role of the alpha-adrenoc regulating noradrenaline overflow by nerve stimulation. *Brit J Pharmacol* 1972. *44* 672-688

FARNEBO, L.-O and B. HAMBERGER, Drug-induced changes in the release of (^{3}H)-noradrenaline stimulated rat iris. *Brit J Pharmacol.* 1971 *43* 97 106.

HOROWITZ, D S. M FOX, III and L. I. GOLDBERG, Effects of dopamine in man *Circulat* *10.* 237 243

IVERSEN L. L. Role of transmitter uptake mechanism in synaptic neurotransmission. Third Memorial Lecture. *Brit J Pharmacol* 1971 *41* 571 591

KIRPEKAR, S. M. and M PUIG, Effect of flow-stop on noradrenaline release from normal sp spleens treated with cocaine, phentolamine of phenoxybenzamine. *Brit J Pharmacol.* 1971 *43*

LANGER, S. Z. The regulation of transmitter release elicited by nerve stimulation through a p feed back mechanism. In *Frontiers in Catecholamine Research* Eds. E. Usdin and S. H. Sny York Toronto Oxford Sydney Braunschweig. Pergamon Press. 1973 543-549

MCDONALD R. H Jr L. I. GOLDBERG, J L. MCNAY and E. P TUTTLE, Jr Effects of dopamin Augmentation of sodium excretion, glomerular filtration rate, and renal plasma flow *J cl* *1964 43* 1116-1124

ROSENBLUM, R., A. R. TAI and D. LAWSON Dopamine in man: Cardiorenal hemodynamics in nor patients with heart disease. *J Pharmacol xp. Ther* 1972. *183* 256-263

STARKE, K., Influence of extracellular noradrenaline on the stimulation-evoked secretion of nor from sympathetic nerves Evidence for an lpha receptor mediated feed-back inhibition of nor release. *Naunyn-Schmiedeberg s Arch Pharmacol* 1972. *275* 11 23

STJÄRNE, L. Frequency dependence of dual negative feedback control of secretion of sympathe transmitter in guinea pig vas deferens. *Brit J Pharmacol* 1973 a. *49* 358-360.

STJÄRNE, L., Rôle of alpha adrenoceptors in prostaglandin E mediated negative feedback cont secretion of noradrenaline from the sympathetic nerves of guinea pig vas deferens. *Pros* 1973 b. *4* 845-851

STJÄRNE, L., Selectivity for catecholamines of presynaptic alpha-receptors involved in feedbac of sympathetic neurotransmitter secretio in guinea pig vas deferens. *Naunyn-Schmiedeber Pharmacol.* 1975. *288* 295-303.

STJÄRNE, L. and K. GRIPE, Prostaglandin-dependent and independent feedback control of nor secretion in vasoconstrictor nerves of normotensive human ubjects: a preliminary report. *Schmiedeberg s Arch Pharmacol.* 1973. *280* 441-446.

WILLEMS, J L. and M. G BOGAERT Th Influence of imipramine on dopamine-induced ganglionic and neurogenic vasodilatation in the dog. *Naunyn-Schmiedeberg s Arch Pharmacol.* 1975 *286*.



Fig. 1. Pressor responses to 3 min periods of ventilation hypoxia between arrows before and after addition of indomethacin (100 μg/ml). Constant volume perfusion of isolated rat lungs. P_{A} is the pulmonary arterial pressure, P_{TP} is the tracheal pressure.

few tests the response gradually increases until there is a maximum effect for the given depth of hypoxia. Several equal maximum responses can then be obtained before a gradual decline in responsiveness occurs. When finally alveolar hypoxia no longer produces a pressor effect, the pulmonary vascular bed will still respond to vasoactive agents, e.g. kallidin. Since we expected inhibitors of PG-synthesis either to have no effect or to reduce the pressor response to hypoxia, we tested the drugs, in all but one case, in periods when the responses were either increasing or were completely stable. Fig. 1 gives an example of the test procedure. First, two control pressor responses to hypoxia were obtained, whereafter indomethacin (2 mg) was added to the perfusate; then the two following pressor responses were recorded.

Fig. 2 demonstrates in diagrammatic form all the experiments. In the left panel Roman numerals I and II indicate the last two responses (ΔP_{A}) obtained before drug administration, whereas III and IV indicate the first two responses obtained afterwards. Zero level in

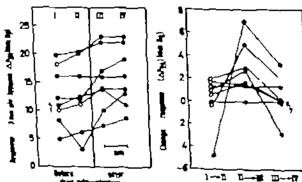


Fig. 2. Left panel demonstrates increases in inflow pressure (ΔP_{PA}) to 3 min periods of ventilation hypoxia in 8 expts.) before (○) and after (●) administration of inhibitors of prostaglandin biosynthesis. Numbers 1 to 8 refers to Table I where the type of drug given to each individual lung preparation is listed. Right panel demonstrates the difference between one hypoxic pressor response and the preceding response ($\Delta(\Delta P_{PA})$). This difference was increased by the administration of prostaglandin biosynthesis inhibitors, which were given between response II and III (◐).

following deep lung inflations (Said, Kitamura and Vreim 1972, Piper and Vaage 1? and during hypoxia (Said and Yoshida 1974)

In order to answer the question whether PGs are mediators of the pulmonary pre response to acute alveolar hypoxia, we have studied the effect of pharmacological inhibi of PG-biosynthesis in an isolated blood-perfused rat lung preparation.

Methods

Experimental preparations. Inbred albino rats (local strain), weighing between 180 and 280 g, were an thetized with pentobarbitone sodium (3–4 mg/100 g b.wt., i.p.) a tracheotomy was performed, and t chest opened during positive pressure ventilation. The trachea, the lungs and the larger intrathoracic sels were dissected free and heparin was injected (100 I U in 0.5 ml saline) into the left ventricle before ligation of the caval veins. Stainless steel cannulas were placed in the pulmonary artery and the left atr The can ulas were fastened by a tight ligature around the heart, and the lung preparation as transfer to a humidified, constant-temperature perspex chamber The lungs were freely suspended in a string t tened to the heart ligature and perfused with 20–25 ml heparinized (10 I U./ml) blood, obtained by car puncture of ether-anesthetized donor rats. At the onset of perfusion flow was adjusted so as to give a m pulmonary arterial pressure ($\bar{P}_{PA}$) of about 12–16 mm Hg. Perfusion was begun within 12 min of the in ruption of the circulation A Harvard Pulsatile Blood Pump (M 1405) givi g constant olume inflow, used P_{PA} was measured by a Statham P23DB pressure transducer connected to a Sanborn Model 33? amplifier recorder via a Sandborn model 350–1100 B DC preamplifier Mean pulmonary arterial press was calculated as the sum of the diastolic pressure and one-third of the pulse pressure. Controls as det mined with electrical damping of the pressure gave corresponding results. The venous effluent as drah l to an open, double-walled thermostated blood reservoir with a bottom outlet. Since left atrial pres and volume flow were kept constant, changes in pulmonary vascular resistance were directly reflected changes in mean inflow pressure (ΔP_{PA}). The method was a modification of that described elsewh (Hauge 1968).

Ventilation After one or two gentle inflations of the lungs constant volume positive pressure ven tion was started using a Starli g Ideal" ventilation pump (C. F Palmer Ltd). End-expiratory pres was kept at bout 2 cm H_2O by means of a water seal. Tracheal pressure was recorded by a differen pressure transducer (Model 270, Hewlett P ckard) connected via a preamplifier to the Sanborn record Stroke olume was adjusted so as to give a peak tracheal pressure of bout 6 cm H_2O at the onset. A fi ventilation frequency of 80 per min was used for all the experiments. The standard gas mixture used f ventilation was 1% O_2, 5% CO_2 and 74% N_2. Alveolar hypoxia was induced by ventilating the lungs a gas mixture of 2% O_2, 5% CO_2 and 93% N_2 for standardized periods of 3 mi with 5 min interv ("hypo ic tests"). The pH of the perfusate was measured with a Radiometer acid–base analyzer (Mo PHM 71).

Drugs. The following inhibitors of PG synthesis were tested Indomethacin sodium meclofenam and acetylsalicylic acid (Vane 1973 Ferreira and Vane 1974).

Indomethacin was prepared as follows. 77.218 g NaH PO was dissolved in 200 ml of sterile water (so tion I). 50 ml of this solution was added to 46.1 ml 1 N NaOH Sterile water was then added up to ? ml (solution II). 85 ml of solutio II were mixed with 2.5 g of indomethacin. 1 N NaOH was added u all indomethacin was solved at pH bout 8.0. Finally solution II was added to a total olume of 100 r The prepared indomethacin solution (25 mg/ml) was stored da k in the refrigerator

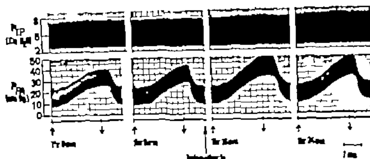
Sodium meclofenamate The necessary amount was made up as required with w ter free of carbon diox

Acetylsalicylic acid ?0 mg acetylsalicylic acid was solved i 1 ml 96 ethanol, and 19 ml of desti water was added.

Kallidin (Synthetic kallidin, Sandoz A.G).

Results

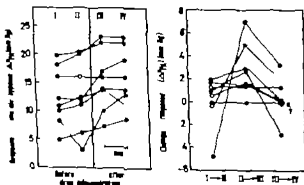
When 3 min periods of alveolar hypoxia are repeated at standardized intervals, the press response in isolated rat lungs follows a characteristic pattern (Hauge 1968 a, b) At the b ... of perfusion such hypoxic tests" produce no or very small pressor responses. Aft



Pressor responses to 3 min periods of ventilation hypoxia between arrows before and after addition meclofenamate (100 μg/ml). Constant volume perfusion of isolated rat lungs. P_{PA} is the pulmonary ar pressure, P_{TP} is the tracheal pressure

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. 2. *Left panel* demonstrates increases in inflow pressure (ΔP_{PA}) to 3 min periods of ventilation hypoxia 8 expts) before (O) and after ( ) administration of inhibitors of prostaglandin biosynthesis. Numbers to 8 refers to Table I where the type of drug given to each individual lung preparation is listed. *Right* el demonstrates the difference between one hypoxic pressor response and the preceding response (ΔP_{PA}). This difference was increased by the administration of prostaglandin biosynthesis inhibitors ich were given between response II and III (●).

TABLE I

Expt. no	Drug (2 mg)	Volume of perfusate (ml)	Flow (ml/min)	Before drug administration			After drug administrat.		
				Baseline P_{PA}	ΔP_{PA} I	ΔP_{PA} II	Baseline P_{PA}	ΔP_{PA} III	[illegible]
1	Acetylsalicylic acid	25		15.3	10.0	11.0	14.8	13.7	[illegible]
2	Indomethacin	21		17.7	12.3	12.0	17.3	17.0	[illegible]
3	Indomethacin	20	14	13.0	18.0	20.0	15.0	23.0	[illegible]
4	Acetylsalicylic acid	20		11.6	10.7	12.4	12.3	13.7	[illegible]
5	Indomethacin	20	10	16.0	8.3	3.0	16.0	10.0	[illegible]
6	Meclofenamate	25		11.6	4.7	6.0	12.0	7.3	[illegible]
7	Indomethacin	22	4	11.3	19.7	20.3	11.3	22.0	[illegible]
8	Meclofenamate	20	16	15.0	16.0	16.0	15.0	16.0	[illegible]

Pulmonary arterial pressure and pressure-changes are given in mm Hg.
Peak rise in P_{PA} during 3 min of ventilation hypoxia.

this panel represents baseline level of P_{PA} before each individual hypoxic test. With the e[xception] of exp. no. 3 Table I the baseline level of P_{PA} did not change. The right hand pa[nel] in Fig. 2 demonstrates *the change* in $\Delta P_{PA}(\Delta(\Delta P_{PA}))$ from I to II, from II to III and f[rom] III to IV. Thus, a continuous increase in the response to hypoxia would give positive val[ues] of $\Delta(\Delta P_{PA})$ and if the increase was linear the values would have been identical. A sudd[en] increase in the response, in excess of a steady linear rise, would have increased $\Delta(\Delta P_{PA})$ compared with the preceding one. Following drug administration this is exactly what ha[p]pened in the majority of preparations. The response to hypoxia was lifted to a higher plate[au]. When all the values of $\Delta(\Delta P_{PA})$ from I to II were regarded as one group and compared [with] the group of $\Delta(\Delta P_{PA})$ from II to III, this last group was significantly increased (P [illegible] Wilcoxon two-sided test). In no case did inhibitors of PG synthesis cause a reduction [of] the pressor response to acute hypoxia. Undiminished responses could be elicited as long [as] 48 min after administration of indomethacin, which is in agreement with the normal re[s]ponse pattern in this preparation.

Table I gives a general overview of preparation parameters such as volume of perfusa[te], flow and baseline inflow pressure (P_{PA}). In addition the table shows which inhibitor dr[ug] that was given to each individual lung preparation and also the numeric values of press[or] responses.

None of the inhibitors of PG-synthesis used had any effect on the vasoconstrictor re[s]ponse to kallidin (100 ng) injected intra-arterially.

Neither hypoxic tests nor the administration of inhibitors of PG biosynthesis caused con[s]istent bronchomotor responses, judged from the observation of tracheal pressure.

Discussion

Inhibition of PG biosynthesis was not experimentally verified in the present study we relied upon the works of other investigators when deciding the dosage of inhibitors the length of the observation periods. The use of nonsteroid anti-inflammatory drugs ool to test the involvement of PGs in biological systems (Ferreira and Vane 1974 b). and Vane (1969) found that 0.1–0.5 μg/ml of indomethacin and 1–5 μg/ml of aspirin ed PG synthesis in isolated guinea-pig lungs perfused with Krebs-Ringer solution. In experiments 2 mg of each agent tested was diluted in about 20 ml of blood, i.e. approximately 100 μg/ml. Since about 90 per cent of indomethacin is bound to plasma-pro- (Vane 1973), this is equivalent to an effective dose of this particular drug of some 10 l, i.e. about 100 times the minimum dose recommended by the above workers. Her -maka-Cedro and Vane (1973) have also shown that renal PG-synthesis *in vivo* was in-ted by indomethacin 2 mg/kg. Plasma protein binding of acetyl salicylic acid is 50–80 cent (Vane 1973). The fraction of meclofenamate which is bound to plasma protein is known (Vane 1973). However when evaluated on a weight basis as well as on a molar s, this drug is the most potent inhibitor of PG-synthesis of the three used in the present k (Ferreira and Vane 1974 a).

Perfused rat lungs have a considerable capacity to release PGs secondary to a wide vari- of stimuli (Bakhle and Vane 1974). Whether intrapulmonary synthesis and release of s will cause a constrictor or dilator response depends on the balance between the dif ent subgroups of PGs which act on vascular smooth muscle cells. $PGF_{2\alpha}$ is a potent lmonary vasoconstrictor agent (Änggård and Bergström 1963), whereas PGE_1 is able to ate pulmonary resistance vessels, provided there is a certain degree of smooth muscle e (Hauge *et al.* 1967). The release of PGs from lungs is dependent on a rapidly induced synthesis, since only negligible amounts of PGs are stored in lung tissue (Piper and Vane 71). Accordingly the present observation that pharmacological blockade of PG synthesis d not diminish the vasoconstrictor response to hypoxia, indicates that vasoconstrictor Gs are not mediators of this response.

An unexpected finding was that nonsteroid, anti-inflammatory drugs sometimes induced moderate potentiation of the vasoconstrictor effect caused by hypoxia. This observation supported, however by a short recent report from Weir *et al.* (1974), in which it is stated at meclofenamate and indomethacin augment the pulmonary pressor response to hypoxia intact dogs. As an explanation it is tempting to speculate whether pulmonary arterial ypertension due to hypoxia might induce synthesis and release of some vasodilatory PGs. PG release has been induced by various chemical and mechanical stimuli with distortion f cell membranes (Piper and Vane 1971). The release of some vasodilatory PGs from lungs uring severe pulmonary hypertension with subsequent moderation of vascular transmural ressure can be regarded as a functional and advantageous phenomenon modulating the esponse to hypoxia. Such a postulated mechanism might then be prevented by administra-ion of PG synthesis inhibitors. A secondary release of this type may be the reason why ther investigators have bserved PG-like biologically active substances in the effluent erfusate from lungs during hypoxia (Said and Yoshida 1974)

Recently Said and coworkers (1974) reported that the hypoxic pressor response of lungs *in situ* is reduced by aspirin. There are, however several reasons why this paper work is difficult to assess. In these experiments aspirin by itself elevated pulmonary vascular resistance (PVR), whereas the PVR-level obtained during hypoxia was unaltered. The reduction in the PVR response to hypoxia was claimed it was based on calculations in per cent of the new PVR base-line levels, assuming a linear system. Furthermore, no tests with a pulmonary vasoconstrictor agent was carried out in order to detect unspecific dampening of vascular smooth muscle reactivity Such general and non-specific depression of vascular smooth muscles has been described for rabbit lungs following the administration of other nonsteroid anti-inflammatory drugs, such as sodium salicylate and phenylbutazone (Hauge *et al* 1966) Finally we would like to point out that also Said and coworkers found that indomethacin (1 to 10 mg/kg) "sometimes even enhanced" the pulmonary arterial pressor response in cats.

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References

ÄNGGÅRD E. and S BERGSTRÖM, Biological effects of an unsaturated trihydroxy acid (PGF$_{2\alpha}$) from normal swine lung. *Acta physiol scand.* 1963. 58 1 12.

BAKHLE, Y S. and J R. VANE, Pharmacokinetic function of the pulmonary circulation. *Physiol* Rev. 1974 54 1007–1045

BERRY P A., J F EDMONDS and J M. WYLLIE, Release of prostaglandin E$_2$ and unidentified factors from ventilated lungs. *Brit J Surg* 1971 58 189–192.

EULER, U S. VON and G LILJESTRAND Observations on the pulmonary arterial blood pressure in the cat. *Acta physiol. scand.* 1946. 12. 301 320.

FERREIRA, S. H and J R. VANE, New aspects of the mode of action of nonsteroid anti-inflammatory drugs. *Ann. Rev. Pharmacol.* 1974 a. 14 57 73

FERREIRA, S. H and J R. VANE, *Aspirin and prostaglandins* in "The prostaglandins" edited by Peter Ramwell, Plenum Press, New York 1974 b. Vol. 2, pp. 1–47

HAAS, F and E. H BERGOFSKY Role of the mast cell in the pulmonary pressor response to hypoxia. *J clin. Invest* 1972. 51 3154–316..

HAUGE, A., Conditions governing the pressor response to ventilation hypoxia in isolated, perfused rat lungs. *Acta physiol. scand.* 1968 a. 72. 33–44.

HAUGE, A., Role of histamine in hypoxic pulmonary hypertension in the rat. I Blockade and potentiation of endogenous amines, kinins and ATP *Circulat R* 1968 b 22 371 383

HAUGE, A P K. M LUNDE and B. A. WAALER, The effect of bradykinin, kallidin and eledoisin upon the pulmonary vascular bed of an isolated blood-perfused rabbit lung preparation. *Acta physiol. scand.* 1966. 66. 269–277

HAUGE, A., P K. M LUNDE and B A WAALER, Effects of prostaglandin E$_1$ and adrenaline on the pulmonary vascular resistance (PVR) in isolated rabbit lungs *Life Sci* 1967 6 673–680.

HAUGE, A. and N C. STAUB, Prevention of hypoxic vasoconstriction in cat lung by histamine releasing agent 48/80. *J appl. Physiol* 1969 26 693–699

HERBACZYNSKA-CEDRO, K. and J R. VANE, Contribution of intrarenal generation of prostaglandin to autoregulation of renal blood flow i the dog. *Circulat Res.* 1973 33 428 36.

KAY J M., J C. WAYMIRE and R. T GROVER, Lung mast cell hyperplasia and pulmonary histamine-forming capacity in hypoxic rats *Amer J* [illegible]

n, G., Discussion remarks to Änggård and Samuelsson: "The metabolism of prostaglandins tissue" in Proc. 2nd Nobel Symp. Prostaglandins, edited by S. Bergström and B. Samuelsson. t & Wiksell, Stockholm 1967 pp. 107–108.
Release and metabolism of prostaglandins in lung tissue. Pol. J. Pharmacol. Pharmacy 1974
2.
. and J. Vane, Unpublished observations. 1973.
and J. R. Vane, Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. Nature (Lond.) 1969 223. 29–37
and J. R. Vane, The release of prostaglandin from lung and other tissues. Ann. N. Y. Acad.
180. 363–385.
S. Kitamura and C. Vertz, Prostaglandin release from the lung during mechanical ventilation tidal volumes. J. clin. Invest. 1972. 51 83a.
and T. Yoshida, Release of prostaglandins and other humoral mediators during hypoxic breathing pulmonary edema. Chest 1974. 66. Suppl. (part 2), 12 A–13 A.
T. Yoshida, S. Kitamura and C. Vertz, Pulmonary alveolar hypoxia: Release of prostaglandins other humoral mediators. Science 1974. 185 1181–1183.
. D. and A. J. Boscha, Histamine-induced pulmonary vasodilatation in the calf: relationship to . J. appl. Physiol. 1973. 35. 830–836.
P. The prostaglandins" edited by P. W. Ramwell. Plenum Press, New York–London, 1973, pp. 203–218.
A. and R. A. Carleton, Prevention of hypoxic pulmonary hypertension by chlorphenhramine. Physiol. 1971 31 531–535.
R., Prostaglandins and Aspirin-like drugs. Pharmacology and the future of man. Proc. 5th Int. Pharmacology, San Francisco 1972, vol. 5. pp. 352–378 (Karger, Basel). 1973.
K., J. T. Reeves and R. F. Grover, Meclofenamate and indomethacin augment the pulmonary response to hypoxia and exogenous prostaglandin $F_{2\alpha}$. The Physiologist 1974. 17 355.

Recently Said and coworkers (1974) reported that the hypoxic pressor response of lungs *in situ* is reduced by aspirin. There are, however several reasons why this particular work is difficult to assess. In these experiments aspirin by itself elevated pulmonary vascular resistance (PVR), whereas the PVR level obtained during hypoxia was unaltered. When reduction in the PVR-response to hypoxia was claimed it was based on calculations in per cent of the new PVR base-line levels, assuming a linear system. Furthermore, no tests with a pulmonary vasoconstrictor agent was carried out in order to detect unspecific damping of vascular smooth muscle reactivity Such general and non-specific depression of vascular smooth muscles has been described for rabbit lungs following the administration of other nonsteroid anti-inflammatory drugs, such as sodium salicylate and phenylbutazone (Hauge *et al* 1966) Finally we would like to point out that also Said and coworkers found that indomethacin (1 to 10 mg/kg) "sometimes even enhanced" the pulmonary arterial pressor response in cats.

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References

ÄNGGÅRD, E. and S. BERGSTRÖM Biological effects of an unsaturated trihydroxy acid (PGF2α) from normal swine lung. *Acta physiol scand.* 1963 *58* 1 12.

BAKHLE, Y S. and J R. VANE, Pharmacokinetic functio of the pulmonary circulation. *Ph siol. Re* 1974 *54* 1007 1045.

BERRY P A., J F EDMONDS and J M WYLLIE, Release of prostaglandin E_2 and unidentified factors from ventilated lungs. *Brit J Surg* 1971 *58* 189–192.

EULER, U S. VON and G LILJESTRAND Observations on the pulmonary arterial blood pressure in the c *A ta physiol scand.* 1946. *12.* 301 320.

FERREIRA, S. H and J R. VANE, New aspects of the mode of action of nonsteroid anti-inflammatory drugs. *Ann Rev. Pharmacol* 1974 a. *14* 57 73

FERREIRA, S. H and J R. VANE, *Aspirin and prostaglandins*, in "The prostaglandins ed ted by Peter W Ramwell, Plenum Press, New York 1974 b. Vol. 2, pp. 1–47

HAAS, F and E. H BERGOFSKY Role of the mast cell in the pulmonary pressor response to hypoxia *clin. Invest* 1972. *51* 3154–3162.

HAUGE, A., Conditions governing the pressor response t ventilation hypoxia in isolated, perfused r lungs. *Acta physiol. scand.* 1968 a. *72* 33–44

HAUGE, A., Role of histamine in hypoxic pulmonary hypertension in the rat. I Blockade and potentiation of endogenous amines, kinins and ATP *Circulat Res* 1968 b. *22.* 371 383

HAUGE, A., P K. M LUNDE nd B. A WAALER, The effect of bradyk nin, kallidin nd eledoisin upon the pulmonary vascular bed of an isolated blood-perfused rabbit lung preparation. *Acta physiol. scand.* 1966. *66* 269–277

HAUGE, A., P K. M LUNDE and B. A. WAALER, Effects of prostagland: E_1 and adrenaline on the pulmonary vascular resistance (PVR) in isolated rabbit lungs *Lif Sci* 1967 *6* 673–680.

HAUGE, A. and N C. STAUB, Prevention of hypoxic vasoconstrictio cat lung by histamine releasing agent 48/80. *J appl. Physiol* 1969 *26* 693–699

HERBACZYNSKA-CEDRO, K. and J R. VANE, Contribution of intrarenal generation of prostaglandin to autoregulation of renal blood flow in the d g. *Circulat Res.* 1973 *33* 428–36.

KAY J M J C. WAYMIRE nd R. T G OVER, Lung mast cell hyperplasia nd pulmonary histamine-forming capacity in hypoxic rats *Amer J.*

der these experimental conditions. This made it of interest to make bioassays in the me species of the antidiuretic activity which could be separated from the urine of goats der normal and stimulated conditions.

Methods

...mals. 8 female goats (b wt. 35 to 40 kg) were used. 2 of the animals were used exclusively for bioassays antidiuretic activity recovered from the urine of the other 6 goats. The animals were routinely confined metabolism cages at room temperature of 20 ± 1°C where they had free access to chaffed hay and water tween the experiments. The goats were maintained in positive sodium balance by addition of 6 g NaCl the 400 g of commercial grain mix they consumed each afternoon.

Ventricular implantations and infusions. Four goats were supplied with permanent cannulae in the lateral rebral ventricle near the foramen of Monro. The three-cannulae system used, and the infusion technique ve been described previously (Åkerlund, Andersson and Olsson 1973). Angiotensin II (0.4 µg/kg min, ypertensin "Ciba") dissolved in hypertonic (0.5 M) NaCl was infused for 30 min periods into the cerebro-inal fluid (CSF) of the lateral ventricle at rate of 10 µl/min.

Hydration and dehydration. Most of the intraventricular infusions were made in the hydrated animal, when water diuresis was fully established. Hydration was accomplished by giving into the rumen by stomach be 100 ml/kg of 38°C water 80 min before an intraventricular infusion was started. The two goats used r bioassays of antidiuretic activity were similarly hydrated during the bioassay experiments. When hydration was used to stimulate ADH secretion, the animals were not given any water to drink for 24 r 48 h before their urine was collected for estimation of antidiuretic activity.

Urine collections and analyses. Urine was collected in 10, 30, 60 or 120 min samples via retention catheter serted into the urinary bladder. Ten min samples were routinely taken in the two goats used solely for ioassays. Urine and plasma [Na^{+}] were determined by use of an EEL flame photometer. An Advanced nstruments Inc. osmometer was used for determinations of urine and plasma osmolality. A plasma smolality round 290 mosm/kg was found during hydration. Hence, this value was used for calculations f the renal C_{H_2O} in bioassays and other experiments performed in hydrated goats.

Adsorption and separation of antidiuretic activity in the urine. The method employed for adsorption and eparation of ADH from the urine as principally the same as those described previously by Ruch (1967) nd by Frandsen (1969). A modification introduced as the column purification with ethanol described elow.

Immediately after collection the pH of the urine was adjusted to 3 with trichloracetic acid (TCA). After cooling and filtration the TCA was extracted ith cooled ethylether and separated. The small amount of thylether remaining in the aqueous phase was removed with nitrogen gas. The pH was then adjusted to 5 ith 2 M NH_4OH and the urine was allowed to pass by its own hydrostatic pressure through resin column (Amberlite IRC 50, XE-64). The diameter of the column was 10 mm and its height 40 mm. After assage of the urine the column was washed twice with 30 ml of redistilled water. T avoid substances hich in preliminary experiments sometimes had been found to obscure the bioassays, the resin column was purified with 10 ml of 50 % ethanol. Finally ADH activity was eluted from the column with 60 ml M 50 HAc at 4°C. The eluate was dried in rotary evaporator at 30°C, and the dry material was stored at 4°C until the time of bioassay. It as then dissolved in 5 or 10 ml of isotonic saline.

Tests are performed in the following way to determine whether the ethanol washing removed any of he ADH activity adsorbed to the resin column. Bioassays were made with eluates of split urine samples, where one half of the sample was eluted from columns not treated with ethanol. These bioassays did not reveal any reduction of the ADH activity eluted from ethanol washed columns. Neither did the ethanol washing of the column reduce the recovery of synthetic vasopressin added to urine before passage through he column. The recovery amounted to 80 of the added hormone.

Trypsin incubation. T eliminate ADH activity in control experiments the dissolved eluate was incubated with trypsin (Trypsin "Novo" 40 µg/ml) at pH 7.8 for 5 hr at 30°C.

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Recovery of ADH Activity in the Urine of Goats under Normal and Stimulated Conditions

By

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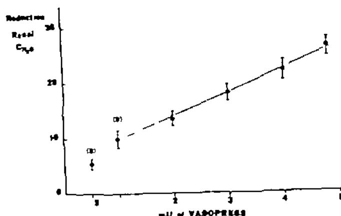
Abstract

LISHAJKO F and B ANDERSSON *Recovery of ADH activity in urine of goats under nor and stimulated conditions* Acta physiol. scand. 1975 95 102–109

The hydrated goat was used for bioassays of antidiuretic hormone (ADH) activity recovered by column separation from the urine of other animals of the same species. A methodological innova- the separation procedure was ethanol purification of the column before elution. In this manner others which sometimes obscured the bioassays were eliminated without loss of ADH activity With the basic method employed, it was possible to determine to the nearest 0.5 of a mU the ADH activity of urine samples, provided they contained between 0–5 mU of ADH. When arginine vasopressin was infused in venously into hydrated animals, slightly more than 10% of its antidiuretic activity was recovered in t urine. In the water replete goat the ADH activity found in the urine was of the order of 1 mU per hr secretion, indicating a neurohypophyseal ADH release of pproximately 5 μU/kg min. After 48 h of dehy tion in an environmental temperature of 20°C the renal ADH excretion increased to about 8 mU/h, sign ing an 8-fold increase of ADH secretion over basic, water replete secretion of ADH. ADH secretion of t same high order was induced in hydrated animals by the infusion of angiotensin II together with hyperto NaCl into the lateral cerebral ventricle.

Over the past several years goats have been used extensively for studies of the centr control of water balance (*cf* Andersson and Olsson 1973) In this research the release antidiuretic hormone (ADH) in response to intracarotid and intraventricular infusions hypertonic NaCl and angiotensin II has been investigated in the hydrated animal. Eviden that the inhibition of the water diuresis elicited by these stimuli in fact is due to rele of ADH has been obtained indirectly It has been shown that the intracarotid and inte ventricular infusions of hypertonic NaCl and angiotensin II no longer elicit an inhibit of the water diuresis when diabetes insipidus has been induced by radio-frequency lesi in the median eminence which interrupt the hypothalamo–neurohypophyseal connections.

The infusion into the third or lateral cerebral ventricles of hypertonic NaCl togeth with angiotensin II appears to be a very potent stimulus for ADH release in the goat, judg from the long-lasting, profound negative renal free water clearance (C_{H_2O}) which develo in the hydrated animal in response to such infusions (Andersson *et al* 1972) However attempt has previously been made to quantitate the amount of ADH which is relea



2. Dose-response curve in one of the bioassay animals to graded amounts of synthetic arginine pressin. See "Methods" for explanation of the way in which the magnitudes of the responses were lated. Figures in brackets – Number of tests which each single dose of vasopressin. Vertical bars ment ±EM.

B. *Recovery of antidiuretic activity in the urine*

Intravenously infused synthetic arginine vasopressin

two goats (hydrated to inhibit their endogenous ADH secretion) intravenous infusions synthetic arginine vasopressin were made at a rate of 1 and 3 mU/min for 30 min. e urine of these animals was collected during the infusions and as long as the vasopressin luced inhibition of their water diuresis lasted. The urine was eluted and bioassays were rformed. In the urine from the goat which received in total 30 mU of vasopressin an antiuretic activity corresponding to 3 mU was recovered. The recovery in the second animal, hich had received in total 90 mU of vasopressin, was 10 mU. Thus, these experiments dicated that 10%, or slightly more, of ADH released into the blood might be recovered y the separation and bioassay methods employed.

. *Water replete and dehydrated goats*

ive water replete goats (n 13) 120 min urine samples were collected for elution and ioassay of ADH activity. The mean plasma osmolality of these animals was 294 mosm/kg range 292 to 296) and their mean plasma [Na] was 145 mEq/l (range 142 to 147). The mean total ADH activity recovered in these 120 min samples was 2 mU (range 1 to 3 mU).

In three of these goats 60 min samples were taken for determination of antidiuretic activity also after 24 and 48 h of dehydration. After 48 h of dehydration the mean plasma osmolality was 305 mosm/kg (range 300 to 310) and the mean plasma [Na] 150 mEq/l (range 148 to 151). In the 60 min samples taken at 24 h of dehydration the recovered mean ADH activity was 3 mU (range 2.5 to 3.5). The corresponding recovery at 48 h of dehydration was 8 mU (range 6 to 9 mU), indicating an 8-fold increase over basic, water replete ADH secretion after two days of total water lack. When offered water at this stage of dehydration the three goats immediately drank between 2 and 3.5 liters of water

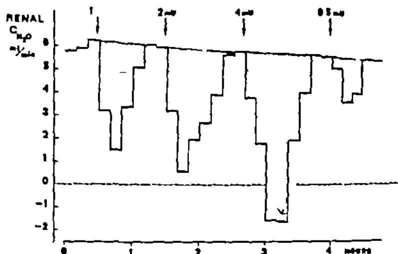


Fig. 1 Antidiuretic responses to graded doses of synthetic arginine vasopressin in one of the two goats used for bioassays of ADH activity recovered from the urine of other goats. Intravenous injection of vasopressin at arrows. C_{H_2O} = Free water clearance.

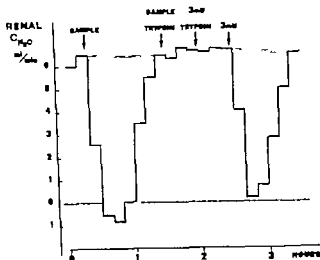
Bioassay method. Each animal was used at the most twice a week for bioassay experiments. After the initial water loading the water diuresis of the two goats reached a plateau within 80 min (renal C_{H_2O} +5 to +7 ml/min). Tests were then started of the antidiuretic response to intravenous injections of exogenous ADH (synthetic arginine vasopressin), and of eluates of urine from other goats. Between the periods of inhibition, the C_{H_2O} approximately returned to the same high, constant level it had at the start of the bioassays. This made it possible to make 4 succeeding tests for ADH activity before the estimated loss of water had to be restored by stomach t be to a old significant decline of C_{H_2O} between tests

Evaluation f bioassay results. For evaluation of the bioassays the renal C_{H_2O} w s plotted as a histogram on millimeter graph paper Hence, any temporary inh bition of the water diuresis appeared as a reduction of the surface area occupied by the C_{H_2O} histogram (Fig. 1). The surface area of this reduction was measured and was compared to the surf ce reduction induced by the i v mjection of a kno amount of arginine vasopressin For simplicity the response to 1 mU of vasopressin was gi en the figure of 10. In the individual a imal, the responses to graded doses of vasopressin calculated in this ma ner formed a rather linear dose-response curve of constant slope within the range 1 to 5 mU of vasopressin (Fig.). Therefore, it was sufficient to make only one or two injections of known amount of vasopressin in each bioassay experiment

Results

A Sensitivity of the bioassay animals to arginine vasopressin

Repeated experiments were performed in both bioassay goats when only their response to graded doses of arginine vasopressin (0 5 up to 5 mU) were studied. A slight and brief (20 to 30 min) reduction of the water diuresis with remaining positive C_{H_2O} was obtained by 0.5 mU of vasopressin (Fig 1), whereas 4 mU invariably induced negative C_{H_2O} lasting for 20 to 30 min. When calculated as described above, the plotted responses to graded doses of vasopressin followed a rather linear curve in the individual animal (Fig. ?). Although some day to day variations in the magnitude of the response to a constant amount of vasopressin were seen the slope of this dose-response curve remained unchanged in experiments performed in the individual animal. Therefore, in subsequent bioassay experiments it was possible to determine to the nearest 0.5 mU the ADH activity of an unknown sample, provided the eluate contained between 0–5 mU of ADH



. Trypsin inactivation of activity recovered from the f goat subjected to central ensin/NaCl stimulation. The samples" initially contained ne ADH activity (about 4 Arginine vasopressin (") was similarly inactivated by n incubation.

e recipient was collected for separation and bioassay of ADH activity An activity mU was recovered, *i.e.* slightly more than 10% of the amount of ADH estimated to been infused intravenously

C. *Trypsin elimination of urinary ADH activity*

ibation with trypsin was employed to obtain further evidence that the antidiuretic vity recovered from the urine exposed true ADH secretion. Intraventricular infusion ngiotensin/NaCl was used to induce antidiuresis in two hydrated goats. The urine ected during the periods of inhibited water diuresis was eluted. Each saline dissolved its was divided in two and one half was incubated with trypsin. Subsequent bioassays ealed ADH activities of 3 and 4 mU in the untreated portions of the eluates, whereas no)H activity remained in the trypsin treated portions. A total disappearance of ADH vity was also obtained when known amounts of synthetic arginine vasopressin were ubated with trypsin in the same manner (Fig. 4).

Discussion

e method initially employed for separation of ADH activity in goats' urine was that scribed by Ruch (1967) without any modifications. However injections of eluates obtained this method occasionally induced sluggishness and a depression of the renal C_{H_2O} of e bioassay animal which apparently was unrelated to the ADH activity of the eluates. uch disturbing side-effects were completely eliminated when ethanol washing of the column as introduced prior to the elution of adsorbed ADH activity It suggests that the ethanol urification of the column removed toxic substances without releasing significant amounts f ADH adsorbed to the column.

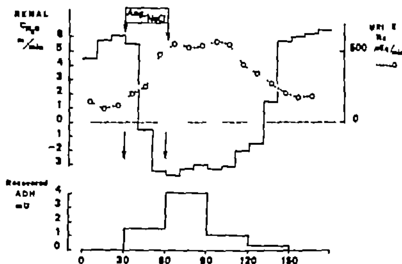


Fig. 3 *Above* Antidiuretic and natriuretic responses of a hydrated goat to the infusion of angiotensin (0 4 ng/kg min) together with hypertonic (0.5 M) NaCl into the lateral cerebral ventricle. Rate of infusion 10 µl/min. *Below* ADH activity appearing in the urine as effect of the intraventricular angiotensin/NaCl infusion. Note No detectable ADH activity in the urine during periods of positive renal free water clearance (C_{H_2O}).

3 *Intraventricular angiotensin /NaCl infusions*

In four hydrated goats angiotensin II (0.4 ng/kg/min) dissolved in hypertonic (0.5 M) NaCl was infused at 10 µl/min into the CSF of the lateral cerebral ventricle for 30 min periods. As expected from previous studies (*cf* Andersson and Olsson 1973), these infusions had strong antidiuretic and natriuretic effects, and induced thirst. However the animals were allowed to take only occasional small sips of water which revealed that the urge to drink remained for about half an hour after cessation of the infusions.

In three experiments the urine collected before, during and after the infusion was pooled in 30 min samples and the antidiuretic activity of each sample was eluted and bio-assayed. No activity was found in the urine secreted during the 30 min preceeding the intraventricular infusion of angiotensin/NaCl An ADH activity of between 1 and 2 mU was recovered in the 30 min sample taken during the infusion, and the recovered activity rose to a mean of 3.5 mU (range 3 to 4) in the first post infusion 30 min period It then declined to roughly 1 mU in the second post-infusion period. No, or very little ADH activity was found in the subsequent 30 min sample which was collected when the renal C_{H_2O} gradually returned to positive values. The results of one of these experiments are presented in Fig. 3

In four identical experiments the urine was collected from the start of the intraventricular angiotensin /NaCl infusion and for 90 min onwards. These four samples were pooled and eluted. One tenth of the eluate was used for bioassay and showed an ADH activity of 3 mU indicating a total mean recovery of 7.5 mU per experiment. The remaining 90% of the eluate (approximate activity ~27 mU) was then infused intravenously over 30 min to a hydrated goat. The infusion induced negative renal C_{H_2O} in the recipient which lasted for 110 min From the start of the infusion and for the entire period of negative C_{H_2O} the urine

References

...on, L. E., I. Andersson and K. Olsson, A cannula system for frequent infusions into the CSF ... cerebral ventricles of the goat. *Physiol. Behav.* 1973. *10*. 161–162.

...on, B., L. Eriksson, O. Fernández, C.-G. Kolmodin and R. Oltner, Centrally mediated effects ... hium and angiotensin II on arterial blood pressure and fluid balance. *Acta physiol. scand.* 1972. ...–407

...on, B. and K. Olsson, On central control of body fluid homeostasis. *Conditional Reflex* 1973. ...–159.

..., I. P. and R. L. Malvin, Plasma concentrations of ADH in conscious and anesthetized dogs. ... *J. Physiol.* 1970 a. *218*. 1128–1132.

..., J. P. and R. L. Malvin, Stimulation of ADH release by the renin-angiotensin system. *Amer. ... Physiol.* 1970 b. *218*. 1555–1559

...n, P. Purification and determination of the antidiuretic substance in human urine. *Acta endocr.* ...) 1949. 42. 31–38.

..., H. D. Metabolism of antidiuretic hormones. *Amer. J. Med.* 1967. 42. 713–744.

... F. S. Identification of the antidiuretic substance in human urine. *Acta endocr.* (Kbh.) 1960. *35* ...74.

... W. Estimation of antidiuretic hormone in the urine of healthy subjects and patients with in... ...propriate secretion of vasopressin (Schwartz-Bartter Syndrome). *Acta endocr.* (Kbh.) 1967. *54*. 113–121

...a, S. K. Motohashi, H. Iba... and S. Ohnaka, Method for the assay of antidiuretic hormone ... with notes on the antidiuretic titer of human plasma. *J. Lab. clin. Med.* 1961. 62. 279–285.

... E., J. A. Johnson and W. W. Moore, Left atrial pressure, plasma osmolality and ADH levels ... unanesthetized ewe. *Amer. J. Physiol.* 1969. *217*. 1672–1680.

Of importance for the interpretation of the present results is the question whether the antidiuretic activity recovered in the urine reflected true secretion of ADH from the neurohypophysis. This seems to have been the case for the following reasons. (1) Eluates of urine collected when the neurohypophyseal release of ADH was inhibited by hydration never had any antidiuretic effect in the bioassay animals. (2) Dehydration, which accelerates the ADH release, markedly increased the antidiuretic activity which could be recovered in the urine. (3) A central stimulus for ADH release (angiotensin in hypertonic NaCl) caused the appearance of large amounts of antidiuretic material in the urine of hydrated goats. (4) Like vasopressin, this antidiuretic material was inactivated by incubation with trypsin. (5) The same degree of urinary recovery was obtained after intravenous infusions of vasopressin, and of antidiuretic material separated from the urine of the goats. Taken together these observations appear to form conclusive evidence that a quantitative measure of renal excretion of ADH was obtained by the methods used in this study.

The fraction of intravenously infused arginine vasopressin which was found to be excreted in the urine of the goats was about 10 %. This value falls within the range (5 to 30 %) previously found in other mammalian species and man (cf. Lauson 1967). Provided the renal excretion of endogenously released ADH is of the same order, the present experiments indicated a basic ADH secretion in the water replete, adult goat of about 5 μU/kg min. Determinations of renal ADH excretion have indicated a basic ADH secretion of similar magnitude (relative to b.wt.) in normal man (Mayer 1960, Ruch 1967).

A 3-fold increase in renal ADH excretion was observed in the goat after 24 h of dehydration at room temperature. After 48 h of dehydration the recovery of ADH activity in the urine was about 8 times higher than in the water replete goat. This relative rise in renal ADH excretion during dehydration in the goat corresponds to the relative increase in plasma ADH level observed during dehydration in man, but is somewhat more pronounced than that observed in the dog and the sheep. Dehydration for 16 h was reported to cause a 3-fold increase of the plasma ADH titre in man ((Yoshida *et al.* 1963) whereas 24 h of dehydration only doubled the plasma ADH titre of dogs (Bonjour and Malvin 1970 a). In sheep dehydrated for 72 h the plasma ADH titre increased by a factor of 4 (Zehr, Johnson and Moore 1969).

It was originally shown by Bonjour and Malvin (1970 b) that angiotensin II stimulates the release of ADH from the neurohypophysis. The intravenous infusion of angiotensin (10ng/kg min) was found to double the plasma ADH titre in the unanaesthetized dog. Later experiments in the goat have revealed that this effect of angiotensin is Na dependent and centrally mediated (cf. Andersson and Olsson 1973). The present study provides a direct confirmation that angiotensin in combination with hypertonic NaCl acts as a very powerful stimulus for ADH release when administered into the cerebrospinal fluid of the lateral cerebral ventricle. In hydrated animals this stimulus induced a urinary excretion of ADH which was of the same magnitude as that observed in the same species after 48 h of dehydration.

... was supported by grants from Karolinsk Inst tutet.

was approached by administration of N^6,2'-O-dibutyryl 3',5'-cyclic AMP (DBcAMP), which has been able to mimic almost any known effect related to an increased intracellular level of cAMP. To exclude unspecific adenosine effects, control experiments with equimolar amounts of 5'AMP (AMP) and adenosine were performed.

Furthermore the effects of DBcAMP, AMP and adenosine on liver function as estimated from the splanchnic elimination of ethanol, the hepatic elimination of Indocyanine Green, and the bile flow were examined. The effects on splanchnic glucose output, uptake or output of lactate, pyruvate and ketone bodies were also investigated.

Methods

[illegible] cats weighing 2.6–4.1 kg and anaesthetized with chloralose (50 mg/kg) and Nembutal (30 mg) were used for the experiments. The methods employed were identical with those previously described (Kravrup [illegible]3 a, b, Kravrup and Larsen 1974).

The body temperature was kept close to 38.5°C by gentle heating. Catheters for infusion, blood sampling and/or pressure recordings were placed in [illegible] femoral artery and vein, [illegible] hepatic vein and/or portal vein. The intra-vascular pressures were measured by means of condensator manometers placed and calibrated at the atrial level. For collection of bile [illegible] catheter was placed in the choledochus after ligation of the cystic duct. A flow probe was placed around the portal vein. When the operation was finished, priming doses of ethanol, (6.5 mmol/kg) and Indocyanine Green (ICG) (300 µg/kg) were given [illegible] femoral vein, followed by continuous i.v. infusions of ethanol (36 µmol/kg/min), ICG (5 µg/kg/min) and taurocholate (0.20 µmol/kg/min).

The cat was left undisturbed for 90 min. Then blood samples from the femoral artery and hepatic [illegible] were drawn at regular intervals for determination of the concentrations of ethanol, ICG, glucose, lactate (L), pyruvate (P), beta-hydroxybutyrate (HB) and acetoacetate (Ac). The oxygen saturation and hemoglobin concentration in arterial, portal venous and hepatic venous blood were also followed. Bile samples were collected for determination of bile flow and biliary ICG excretion. After [illegible] control period of [illegible] minutes, DBcAMP, cAMP, AMP or adenosine were infused, and blood samples were drawn with the same intervals during another 60 min period.

Administration of DBcAMP, cAMP, AMP and adenosine

[illegible] preliminary series DBcAMP was infused into the portal vein in doses ranging from 10 to 1000 nmol/kg/min, and cAMP was administered in doses up to 1200 nmol/kg/min. In the rest of the experiments DBcAMP was administered at doses of 85 nmol/kg/min (n = 3), 340 nmol/kg/min (= 8), and [illegible]0 nmol/kg/min (n = 3). In half of these experiments DBcAMP was infused into the portal vein, in the remaining into the femoral vein. The preparations used were commercial products from Boehringer Mannheim.

Calculations

The estimated hepatic blood flow (EHBF) was determined by means of ICG using the Fick principle. The hepatic arterial blood flow was estimated by subtraction of the portal venous flow from the EHBF. The vascular conductances were calculated in the following manner:

GIC = GIF/BP − PP ml kg^{-1} min^{-1} mmHg^{-1}

HAC = HAF/BP ml kg^{-1} min^{-1} mmHg^{-1}

PVC = GIF/PP ml kg^{-1} min^{-1} mmHg^{-1}

(GIC, HAC, PVC: conductances in the gastrointestinal area, hepatic artery and intrahepatic portal venous bed, respectively. GIF: gastrointestinal (= portal venous) blood flow. HAF: hepatic arterial blood flow. BP: mean arterial blood pressure. PP: portal venous pressure). DBcAMP did not influence the hepatic venous pressure in [illegible] few experiments, and the measured values corresponded to previous findings in our laboratory (Kravrup 1973 b). Therefore the hepatic venous pressure was set to zero in the calculation of PVC.

[illegible]

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From the Institute of Physiology University of Aarhus, Denmark

Imitation of Glucagon Effects on Splanchnic Hemodynamics and Liver Function by N⁶,2′-O-Dibutyryl 3′,5′-Cyclic AMP (DBcAMP) in Cats

By

NIELS KRARUP JENS ANKER LARSEN and ANDERS MUNCK

Received 18 March 1975

Abstract

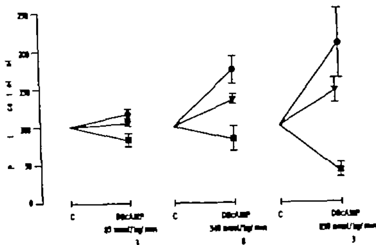
KRARUP N., J A LARSEN and A. MUNCK. *Imitation of glucagon effects on splanchnic hemodynamics and liver function by N⁶,2′-O-dibutyryl 3′,5′-cyclic AMP (DBcAMP) in cats* Acta physiol. scand 1975 95 110–116

Fasting cats anesthetized with chloralose were used for the experiments. DBcAMP infused at a rate of 340 nmol/kg/min increased the gastrointestinal and intrahepatic portal conductances whereas the hepatic arterial conductance was decreased. The hemodynamic responses to portal and systemic venous administration of DBcAMP were identical. In half of the experiments DBcAMP increased the splanchnic ethanol elimination rate and oxygen consumption and in all experiments there was a decrease in the plasma clearance and extraction ratio of Indocyanine Green No change in bile flow was observed. DBcAMP infused at a rate of 85 nmol/kg/min was without significant effects on either splanchnic hemodynamics or liver metabolism. DBcAMP infused at a rate of 850 nmol/kg/min accentuated the decrease in hepatic arterial conductance but was found to decrease the splanchnic ethanol elimination rate and oxygen consumption. Infusion of cAMP AMP and adenosine at a rate of 340 nmol/kg/min were without measurable effects. Based on these results it is concluded that like the metabolic effects also the vascular effects of glucagon are caused by stimulation of specific glucagon receptors which results in an intracellular release of cAMP

Administration of glucagon to cats exerts a marked vasodilatation in the gastrointestinal area which is independent of the changes in hepatic metabolism caused by the hormone (Krarup and Larsen 1974)

It is generally accepted that the metabolic effects of glucagon is caused by liberation of intracellular cyclic AMP (cAMP) according to the second messenger concept (Sutherland and Robinson 1966, Exton *et al* 1971) It has also been shown that the relaxing action of different agents on isolated vascular smooth muscle is mediated via cAMP (Andersson 1973) It might therefore be expected that the gastrointestinal vasodilatation caused by glucagon is due to an increased level of cAMP in the smooth muscle cells of the gastrointestinal resistance vessels.

The aim of the present study was to examine whether the demonstrated effects of glucagon on hepatosplanchnic hemodynamics could be explained by the second messenger concept



The effects of different infusion rates of DBcAMP on gastrointestinal, portal venous and hepatic conductances. (indicates control period, O gastrointestinal conductance, ∇ portal venous conductance, □ hepatic arterial conductance.)

...osplanchnic hemodynamics

...fusion of DBcAMP at a rate of 340 nmol/kg/min caused a small, but not significant ...n arterial blood pressure (from 147±8 to 158±9). The portal venous flow increased ...y after the beginning of the DBcAMP infusion and reached a new steady level about ... higher than the control level in the course of 10 min. In contrast the estimated hepatic ...al flow was slightly but not significantly decreased by DBcAMP. Portal pressure ...ased to a smaller degree than portal flow. DBcAMP therefore increased the gastroin...al and intrahepatic portal conductances, whereas hepatic arterial conductance was ...eased (see Fig. 2). The hemodynamic responses to portal and systemic venous admin...tion of DBcAMP were identical. When the infusion was stopped, the portal venous ... declined to the control value after 10–15 min.

...fusion of DBcAMP at rate of 85 nmol/kg/min (n = 3) had no significant effect on the ...odynamic parameters followed. DBcAMP infused at a rate of 850 nmol/kg/min (n = 3) ...ed a decrease in hepatic arterial conductance, whereas no further increase in gastro...stinal and portal venous conductances occurred. The results are illustrated in Fig. 2. ...AMP and adenosine infused at rate of 340 nmol/kg/min caused no significant changes ...epatosplanchnic hemodynamics.

...er function and metabolism

... half of the experiments in which DBcAMP was given at a rate of 340 nmol/kg/min the ...tial elimination rate of ethanol was low and increased 22% (from 27±2 to 33±2 μmol/ .../min (p 0.001)) together with a 38% increase in hepatic oxygen consumption (from ...±6 to 66±8 μmol/kg/min (p < 0.05). In 3 cats in which the initial elimination rate of ...hanol was larger (33 μmol/kg/min) DBcAMP did not influence the splanchnic ethanol ...imination rate and the hepatic oxygen consumption. In none of the experiments was any

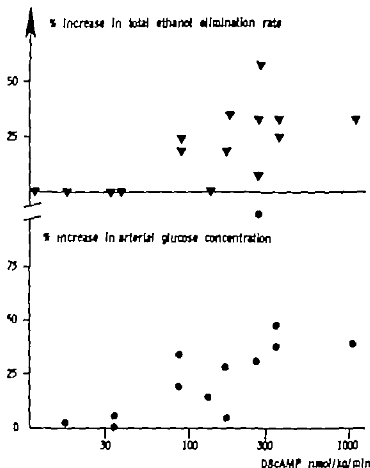


Fig. 1 The effects of vario fusion rates of DBcAMP on t ethanol elimination rate and terial glucose concentration.

The total elimination rate of ethanol was calculated as the amount infused per minute corrected for amount disappeared from r retained in the solvent space set as 65% of the body weight (Larsen 19 The splanchnic uptake or output of the different substances were calculated from the arterio-hep venous concentration gradients and the EHBF The hepatic oxygen consumptio was determined fr the difference between total splanchnic and gastrointestinal oxygen consumption. The clearance and traction ratio of ICG was determined by dividing the amount eliminated respectively the arte hepatic venous difference by the arterial plasmaconcentration at equilibrium.

Statistical procedure

The significance levels were determined by the method of paired comparison using the Student's t-test

Results

In 5 preliminary expts. with cAMP given at doses up to 1 200 nmol/kg/min no hemody mic or metabolic changes could be demonstrated In a preliminary series of 15 expts. t dose-response relationship of DBcAMP was examined The effects of various infusion ra on total ethanol elimination rate and arterial glucose concentration are illustrated in Fig. In 5 expts. in which the infusion rates varied from 130–340 nmol/kg/min, an increase portal flow of about 50% was observed. Based on these results an infusion rate of 340 nm kg/min was chosen for further examination of the effects of DBcAMP

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From the Department of Medical Chemistry University of Helsinki, Finland

Accumulation of Calcium by Retinal outer Segments

By

KARI HEMMINKI

Received 18 March 1975

Abstract

HEMMINKI, K. *Accumulation of calcium by retinal outer segments.* Acta physiol. scand. 1975. 95 117–125

preparations of bovine photoreceptors, including intact and lysed outer segments, are used to study mulation of Ca^{++} in order to differentiate between binding and transport. The two preparations acmulate rapidly roughly equal amounts of Ca^{++} in the presence or absence of ATP. In the absence of P no further accumulation occurs. With ATP accumulation continues most rapidly in intact outer segts. The addition of Ca^{++}-ionophore (A 23187) to assays containing ATP results in kinetics of upsimilar to those in the absence of ATP. Thus the initial phase of Ca^{++} accumulation is predominantly to binding, while that induced by ATP is due to transport. Accumulation of Ca^{++} by outer segments be stimulated by preloading them with ATP suggesting that ATP enters the structures and acts intracellarly. Illumination decreases binding of Ca^{++} to outer segments but does not consistently affect transport. After illumination more Ca^{++} is retained by intact than lysed outer segments indicating that the membrane acts as barrier to the escape of Ca^{++}. Phospholipids and protein appear to be responsible for Ca binding of outer segments as suggested by reduced binding ability after removal these constituents. Phosphatidyl ethanolamine is the main phospholipid class of outer segments.

lcium ions have been suggested to mediate between absorption of light and hyperpolarization of the cell membrane in retinal photoreceptors (Tomita 1970, Hagins 1972). Evidence is now accumulating in support of the involvement of Ca in light-induced changes. Ca^{++} has been reported to be released from outer segments on illumination (Mason et al. 1974 Hemminki 1975) and some data suggest that accumulation of Ca^{++} by outer segments is regulated by light (Mason et al. 1974).

Accumulation of Ca^{++} ions by photoreceptors is stimulated by exogenous ATP (Bownds et al. 1971 Neufeld et al. 1972) but it has not been established whether ATP increases binding or transport of the ion. In this study accumulation of Ca^{++} by intact and lysed bovine outer segments is investigated. A calcium ionophore, A23187 (Caswell and Pressman 1972) is applied in the separation of binding and transport. Regulation of accumulation of Ca^{++} by light is investigated and the Ca^{++} binding component is characterized.

References

ANDERSSON, R Cyclic AMP as a mediator of the relaxing action of papaverine, nitroglycerine, &c and hydralazine in intestinal and vascular smooth muscle. *Acta pharmacol.* (Kbh.) 1973. 32 33 \
DOBBS, L. W and G A. ROBINSON, Functional biochemistry of beta receptors in the uterus. *Fed* / 1968. 27 352.
EXTON, J H G A. ROBINSON, E. W SUTHERLAND and C. R. PARK, Studies of the role of ade 3 -5 -monophosphate in the hepatic actions of glucagon and catecholamines. *J biol. Chem* 197 6166–77
HENION, W F E. W SUTHERLAND and TH. POSTERNAK Effects of derivatives of adenosine 3',5' phate on liver slices and intact animals. *Biochem. biophys. Acta* (Amst.) 1967 148. 106–13.
KAUKEL, E. and H. HILZ, Permeation of dibutyryl cAMP into Hela cells and its conversion to butyryl cAMP *Biochem. biophys. Res. Commun* 1972. 46 1011 19
KRARUP N The effect of noradrenaline and adrenaline on hepatosplanchnic hemodynamics, func capacity of the liver and hepatic metabolism. *Acta physiol. scand.* 1973 a. 87 307 19
KRARUP N The effect of hemorrhage on hepatosplanchnic hemodynamics, liver function and h metabolism. *Acta physiol scand.* 1973 b. 89 269–77
KRARUP N and J A. LARSEN The effect of glucagon on hepatosplanchnic hemodynamics, func capacity and metabolism of the liver in cats. *Acta physiol. scand.* 1974. 91 42 52.
KRARUP N J A. LARSEN and A. MUNCK, The effects of glucagon and dibutyryl cyclic AMP hepatosplanchnic metabolism and hemodynamics in cats. *Digestion* 1974. 10 No. 4–5. 361
LARSEN, J A., Elimination of ethanol as a measure of the hepatic blood flow in the cat. *Acta physiol. sc* 1963 57 209–23
LARSEN, J A and N KRARUP Relation between the hepatic elimination rate of ethanol and molecular respiration, Alfred Benzon Symposium VI Munksgaard Copenhagen 1974 434–42.
MEHANAN, L. A. and O WIELAND Glucagon-like action of N⁶,2 -O-dibutyryl cyclic 3 ,5 -AMP in perfused rat liver *Biochem biophys. Res. Commun.* 1967 29 880–85.
MOORE, P F L. C. IORIO and J M McMANUS, Relaxation of the guinea-pig tracheal chain prepar by N⁶,2 -O-dibutyryl 3 5 -cyclic adenosine monophosphate. *J Pharm. Pharmacol.* 1968. 20. 368–
SUTHERLAND E. W and G A. ROBINSON, The role of cyclic 3 ,5 AMP in responses to catecholamines and other hormones. *Pharmacol. Rev* 1966. 18 145–61

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...preparations of bovine photoreceptors, including intact and lysed outer segments, are used to study ...mulation of Ca^{++} in order to differentiate between binding and transport. The two preparations ac...mulate initially roughly equal amounts of Ca^{++} in the presence or absence of ATP. In the absence of ... no further accumulation occurs. With ATP accumulation continues most rapidly in intact outer seg...ts. The addition of Ca^{++}-ionophore (A 23187) to assays containing ATP results in kinetics of up... similar to those in the absence of ATP. Thus the initial phase of Ca^{++} accumulation is predominantly ... to binding, while that induced by ATP is due to transport. Accumulation of Ca^{++} by outer segments ... be stimulated by preloading them with ATP suggesting that ATP enters the structures and acts intra...larly. Illumination decreases binding of Ca^{++} to outer segments but does not consistently affect ...sport. After illumination more Ca^{++} is retained by intact than lysed outer segments indicating that ... membrane acts as barrier to the escape of Ca^{++}. Phospholipids and protein appear to be ...sponsible for Ca^{++} binding of outer segments as suggested by reduced binding ability after removal ... these constituents. Phosphatidyl ethanolamine is the main phospholipid class of outer segments.

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Materials and Methods

Isolation f outer segments

Bovine eyes were removed immediately after slaughter and placed in a light-proof container at 1 for transportation. After about 1 h retinas were dissected under dim red light, washed in 0.25 M and collected in 1.0 M sucrose (0.5 ml/retina at 0°C). The suspension of retinas was shaken by 2 15 s and transferred into a beaker which was covered with a nylon mesh (hole size 0.5 mm liquid was sieved through the mesh and the contents of the beaker were washed with 0.5 M sucro ml/retina) the wash was filtrated through the mesh and combined with the first slurry The m was centrifuged at 9000 g for 10 min and the supernatant was decanted. The pellet was resuspe 1.05 M sucrose (1.5 ml/retina) and transferred into cellulose nitrate tubes. 5 ml of 0.85 M sucro overlayered on 25 ml of 1.05 M sucrose containing the sample and the gradient was centrifuged in SW 25 rotor at 63000 g for 30 min. The interfacial layer was collected with a Pasteur pipette, dilu 3 vol. of 0.25 M sucrose and sedimented at 9000 g for 10 min. The pellets contained retinal outer Outer segments were lysed by maintaining in distilled water for 10 min. The extent of lysis was by injecting samples of outer segments (0.1 ml) into 0.12 M ammonium acetate (1 ml) and rapidly me the change of absorbance at 640 nm.

Procedure

Ca^{++} accumulation was assayed as described recently (Hemminki, 1974) by incubating samples of o segments (50 μl) in 'physiological medium containi g 0.12 M NaCl, 10 mM KCl, 1 mM $MgSO_4$, 1 $CaCl_2$ and 10 mM sodium phosphate buffer pH 7.4 $^{45}CaCl_2$ (s.a. 10-40 mCi/mg Ca^{++} Amersham used at 0.5 μCi. Incubations were performed in room light at 37°C. Incubations were terminated by a 3 ml of 0.1 M Tris-HCl, pH 7.5 and transferring the mixtures onto Millipore filters (0 45 μm), pre soaked in 0.25 M KCl. The membranes were washed with 2 4 ml batches of Tris with ... and counted in 5 ml of Bray s scintillation fluid. Blanks contained no outer segments. A calcium ionop A 23187 (Cashwell and Pressman 1972 Foreman *et al.* 1973), was a gift from Eli Lilly

Purity of outer segments was investigated by assaying for marker enzymes including cholinest (E.C. 3 1 1.8), a marker of neuronal membranes, succinate dehydrogenase (E.C. 1.3.99 1), a mitochon marker and rotenone-insensitive NADH-cytochrome C oxidoreductase (E.C. 1 6.99.3) a microsomal ker The enzyme assays are described elsewhere (Hemminki and Suovaniemi 1973).

Phospholipids were extracted by the system of Folch and analysed as described previously (Hem 1973). Cholesterol was determined according to Pearson *et al.* (1953).

Electron microscopy was performed according to Sabatini *et al.* (1963).

Results

Characterization of outer segments

Intact bovine retinal outer segments were prepared by gradient centrifugation and for preservation of structure they were kept in isotonic or for short times, in hypertonic tions. Outer segments of this preparation appeared intact and were surrounded by un rupted plasma membranes (Fig. 1). Lysed outer segments (Fig. 2) were prepared by susp ing outer segments in water The fraction composed of vesicularized membranes. The de of lysis of outer segments was checked by an osmotic test. Outer segments were suspende ammonium acetate, which passes freely into these structures causing osmotic swelling (ler *et al.* 1971) These results are shown in Table I Absorbances of the fractions (1 protein) were 1.26 and 0.03 in intact, and lysed outer segments, respectively In good a ment with the electron microscope observations.

Purity of outer segments was examined with marker enzymes and spectral ratios (T II) Markers of neuronal and microsomal membranes showed high purity of outer segm





1 Fig 2

1 An electron micrograph of intact bovine retinal outer segments, 6 000.

2 Lysed outer segments, prepared by maintaining outer segments in water for over 10 min, 3 900.

he specific activities of these enzymes in the samples were only 7% of the homogenate vities.

No mitochondrial contamination was detected in outer segments. The spectral ratios in-ted concentration of rhodopsin by 6.5 to 10-fold which is among the best purifications d in the literature (Zorn and Futterman 1973 Daemen 1973).

mulation of calcium by outer segments

effect of added ATP on the accumulation of calcium was studied with intact and lysed er segments (Fig. 3) At 1 mM concentration ATP increased the initial accumulation of a only slightly within 30 min a 4-fold increase was observed with intact outer segments compared to a less than 2-fold increase with lysed outer segments. As lysed outer seg-nts are osmotically inactive, they are likely to lack transport functions. Thus ATP may hance a transport mechanism for Ca++ rather than facilitate binding of the ion.

In order to differentiate between binding and transport a calcium ionophore A 23187 ashwell and Pressman 197?) was used (Fig. 4). Kinetics of Ca++ uptake in the presence

ble I. Osmotic properties of intact and lysed bovine outer segments. Samples were mixed with 0.12 M ammonium acetate and absorbance was monitored at 640 nm

	Δ absorbance/mg protein
tact outer segments	1.26
ysed outer segments	0.03

TABLE II Enzymatic and spectral characterization of lysed retinal outer segments. Enzyme activities expressed in nmol/min/mg protein. Means ±S.E. of 4 to 5 determinations.

	A Outer segments	B Homogenate	B/A
Cholinesterase	2.1±0.8	29.3±2.2	14.0
Succinate dehydrogenase	0	49.2±2.9	∞
Rotenone-insensitive NADH cytochrome C reductase	8.0±2.4	124±10.1	15.5
A278/A498	2.24±0.07	14.5±0.7	6.5
A400/A498	0.294±0.04	3.01±0.07	10.3

of A 23187 were largely similar to those observed in the absence of ATP (Fig. 3 A). Net accumulation of Ca++ occurred after 1 min. It therefore appeared likely that the initial uptake was largely due to binding of Ca while the delayed uptake, induced by ATP would be due to transport. If it is assumed that A 23187 makes membranes of outer segments freely permeable to Ca++ Fig. 4 B would then describe the maximal Ca++ binding capacity of the membranes about 5 nmol Ca++/mg protein. It is of interest to refer to Fig. 3 A showing that even intact outer segments acquire about 50% of the maximal binding capacity within 1 min This indicates ready accessibility of a number of Ca+ binding sites.

Accumulation of Ca++ by outer segments was studied at 2 different Ca++-concentrations 1 mM and 4×10⁻⁴ M as shown in Fig. 5 At low Ca concentrations the kinetics were largely similar to those at the high concentration of Ca

Stimulation of Ca uptake by outer segments, described in Fig. 3 was suggested to reflect stimulation of transport It was therefore of interest to show whether ATP entered outer segments. Outer segments were incubated in the presence of ATP at 0°C for 30 min and assay for Ca++ uptake was performed. If the uptake was stimulated as compared to the control samples, where ATP was added immediately before the assay this would then suggest that ATP slowly diffused into outer segment This appeared to be the case (Table III); uptake was stimulated by a factor of 1 3 in intact outer segments. The stimulation of transport was possibly even higher since binding, as measured in the presence of the calcium ionophore was somewhat lower in the experimental samples. These data suggest that ATP stimulates transport of Ca++ into outer segments intracellularly

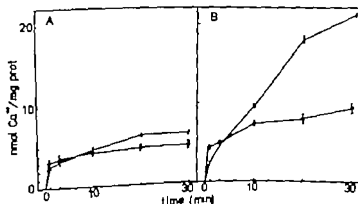
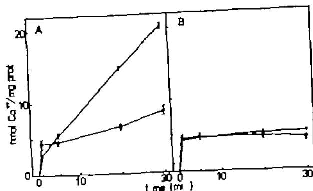
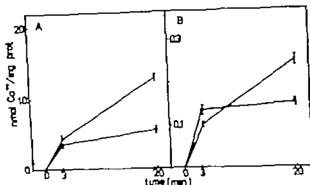


Fig. 3. Effect of ATP on the accumulation of radioactive calcium to retinal outer segments. Outer segments containing about ... of protein were incubated in physiological medium containing ... μCi ⁴⁵CaCl₂ in total volume 450 μl. In (B) 1 mM ATP included. (●—) intact outer segments, (—○—) lysed outer segments. Means ±S.E. of 4 ...



4. Effect of A 23187 cal- ionophore, on the accumula- of radioactive calcium into al outer segments. Incubation ditions as described in Fig 3. assays were performed in the ence of 1 mM ATP (B) con- ed 0.9 μM A 23187 (—●—) ct outer segments, (—○—) d outer segments. Means ± . of 4 expts.



. 5. Effect of calcium concen- ion on the accumulation of ioactive calcium in retinal outer ments. (A) was incubated in siological medium containing M $^{45}CaCl_2$ and 0.5 μCi $^{45}CaCl_2$ ile in (B) non-radioactive calc- was omitted. The incubations e performed in the presence of mM ATP (—●—) intact outer ments (—○—) lysed outer seg- nts. Means ± S.E. of 4 expts.

fect of illumination on the accumulation of calcium

ght regulation of Ca^{++} accumulation by outer segments was investigated by incubating minated and unilluminated preparations in the presence of radioactive Ca^{++} (Table IV). cubation conditions were such that mainly binding of Ca^{++} was probed, *e.g.* absence of TP and presence of A 23187. Illuminated preparations bound consistently less calcium. hen the assays were carried out in conditions suitable to monitor transport, no light regu-

ABLE III Accumulation of calcium by outer segments preloaded with ATP. Outer segments were pre- incubated in physiological medium with or without 2 mM ATP at 0°C in the dark. After 30 min 2 mM ATP was added to the control samples; 0.5 μCi of $^{45}CaCl_2$ and in indicated cases 1 μM A 23187 were added. Samples were incubated at 37°C for 10 min and collected on filters. Means ± S.E. of 8 determinations. Significant at $p < 0.05$ in two-tailed Student's t-test.

	Calcium binding, nmol Ca^{++}/mg protein	
	Preincubation with ATP	Preincubation without ATP
ntact outer segments	17.4 ± 0.8	13.6 ± 0.5
A23187	4.20 ± 0.24	7.42 ± 0.88
ysed outer segments	6.92 ± 0.41	6.25 ± 0.22
A23187	4.31 ± 0.29	4.88 ± 0.32

TABLE II Enzymatic and spectral characterization of lysed retinal outer segments. Enzyme activities expressed in nmol/min/mg protein. Means ±S.E. of 4 to 5 determinations.

	A Outer segments	B Homogenate	B/A
Cholinesterase	2.1±0.8	29.3±2.2	14.0
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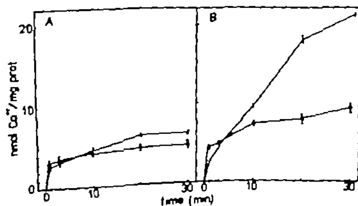


FIG. 3 Effect of ATP on accumulation of radioactive into retinal outer segme segments containing abo of protein were incubated in physiological medium containing μC $^{45}CaCl_2$ in a total ob 450 μL. In (B) 1 mM ATP included (—●—) intact outer ments, (—○—) lysed outer ments. Means ±S.E. of 4

…LE VI. Effect of modifications on the binding of calcium to outer segments. Phospholipid extraction was performed by suspending outer segments on ice in 90% acetone containing 10% water and 0.01% NH_3 for 10 min. The samples were centrifuged at 40 000 g for 10 min and washed twice with 0.1 M Tris, pH 7.4. Means of 3 determinations.

…atment	Ca^{++} binding/mg protein % of control	
	At 5 10^{-6} M $CaCl_2$	At 10^{-4} M $CaCl_2$
…ntrol	100	100
…nase, 100 µg/ml	41	35
…ospholipid extraction	41	39

…-75), then phospholipids appear to be responsible for about 60% of Ca^{++} binding of the …gh and the low affinity sites. Interpretation of the effect of pronase treatment is more …mbiguous since the data is calculated per unit protein and pronase reduces the amount of …otein. It appears, however that protein could be responsible for the remaining Ca^{++} …nding capacity 40% of the total. Neuraminidase treatment was also applied but this …iled to reduce the Ca^{++} binding properties of outer segments.

…Phospholipids were quantitated in detail as they were shown to be the major Ca^{++} bind…ng component of outer segments. Phospholipid/protein ratio of outer segments was 0.58 (Table VII) and the major phospholipid class was phosphatidyl ethanolamine constituting … 7% of all phospholipids.

Discussion

…olated photoreceptor membranes have been shown to take up calcium, which process is …imulated by ATP (Bownds *et al.* 1971 Neufeld *et al.* 1972) Accumulation of calcium by …hotoreceptors has since been investigated by a number of workers (Yoshikami and Hagins …973, Mason *et al.* 1974, Hemminki 1975) but, it has remained unestablished, whether the …rocesses investigated have been due to binding or transport of calcium.

In this study highly pure preparations of bovine outer segments, intact and lysed, were …sed to differentiate between binding and transport of calcium. The intact and lysed pre…arations accumulated equal amounts of calcium in the absence of ATP (Fig. 3 A). This is … strong argument against the possible contribution of mitochondria to the Ca^{++} accumula-

…ABLE VII. Lipid composition of outer segments. Means of 2 to 4 determinations.

…holesterol/protein (w/w)	0.044
…hospholipid/protein (w/w)	0.58
phosphatidyl ethanolamine (%)	42.7
phosphatidyl choline	36.6
phosphatidyl serine	15.3
phosphatidyl inositol	1.3
sphingomyelin	1.2
phosphatidic acid	0.8
lysophosphatidyl choline	1.8
lysophosphatidyl ethanolamine	0.3

TABLE IV Effect of light on the binding of calcium to outer segments. Aliquots of outer segments were illuminated in room light for 5 min and incubated with unbleached controls in the dark 15 min as described in Fig. 3 Means ±S.E. of 4 determinations. Significant at p 16

	Calcium binding, nmol Ca^{++}/mgprotein		
	Dark (A)	Light (B)	A/B 100
Incubation without A 23187			
Intact outer segments	5.23±0.28	4.34±0.19	120
Lysed outer segments	4 72±0 08	3 63±0.24	130
Incubation with A 23187			
Intact outer segments	4 13±0.11	2.89±0.21	141
Lysed outer segments	4.03±0.30	2.84±0.12	141

lation was observed (not shown) These results suggest that binding rather than transp of Ca^{+} is responsive to illumination in bovine outer segments.

In another series of experiments outer segments were first loaded with radioactive C by incubating them with $^{45}CaCl_2$ in the dark. Aliquots of outer segments were then expo to room light and the radioactivity retained was determined (Table V). No change was served in the amount of Ca^{++} in intact outer segments. By contrast, unbleached preparat of lysed outer segments bound more Ca than the illuminated samples. This may sug that intact plasma membranes serve as a barrier to Ca^{++} released from discs.

Characterization of calcium binding components

It is possible to analyse which components of the disc membrane are responsible for C binding by a selective removal of substances with known Ca^{++} binding properties (H minki 1974). The assays were performed at two Ca^{++} concentrations to probe the high the low affinity binding sites of outer segments (Hemminki 1975) Two membrane modi tions tested produced marked decrease in Ca binding to outer segments digestion pronase and extraction of phospholipids (Table VI) Each of the two reduced Ca bin by about 60%. If it is assumed that extraction of phospholipids is complete (see Hem

TABLE V Effect of light on the retention of calcium by outer segments preloaded with $^{45}CaCl_2$. A pension of outer segments (1 mg protei in 400 µl of 0.5 M sucrose) was incubated ith $^{45}CaCl_2$ t 37° in the dark for 15 min. 15 ml of 0.25 M sucrose were added nd the sampl centrifuged at 20 000 g for 15 min. The pellet was suspended in 1 6 ml of 0.5 M sucro divided in two. One half was maintained in the dark and the other half was illuminated in light for 5 min. Aliquots of 250 µl were collected on Millipore filters and analysed as des in Materials and Methods. Means ±S.E of 6 determinations. Significant at $p<0.05$ in tailed Student t-test.

	Calci m retained nmol Ca^{++}/mg protein		
	Dark (A)	Light (B)	A/B 100
Intact outer segments	0.835±0.026	0.848±0.049	99
Lysed outer segments	0 613±0 0.4	0.513±0.030	1?0

...VI. Effect of modifications on the binding of calcium to outer segments. Phospholipid extraction was performed by suspending outer segments on ice in 90 % acetone containing 10 % water and 0.01 M NH_3 for 10 min. The samples were centrifuged at 40 000 g for 10 min and washed twice with 0.1 M Tris, pH 7.4. Means of 8 determinations.

...tment	Ca++ binding/mg protein % of control	
	At 5 · 10^{-5} M $CaCl_2$	At 10^{-4} M $CaCl_2$
...trol	100	100
...ase, 100 µg/ml	41	35
...pholipid extraction	41	39

...5), then phospholipids appear to be responsible for about 60 % of Ca^{++} binding of the ...h and the low affinity sites. Interpretation of the effect of pronase treatment is more ...biguous since the data is calculated per unit protein and pronase reduces the amount of ...tein. It appears, however that protein could be responsible for the remaining Ca^{++} *...nding capacity 40 % of the total. Neuraminidase treatment was also applied but this* ...ed to reduce the Ca^{++} binding properties of outer segments.

Phospholipids were quantitated in detail as they were shown to be the major Ca^{++} bind-...ent component of outer segments. Phospholipid/protein ratio of outer segments was 0.58 ...ble VII) and the major phospholipid class was phosphatidyl ethanolamine constituting ...7 % of all phospholipids.

Discussion

...olated photoreceptor membranes have been shown to take up calcium, which process is ...mulated by ATP (Bownds *et al.* 1971 Neufeld *et al.* 1972). Accumulation of calcium by ...otoreceptors has since been investigated by a number of workers (Yoshikami and Hagins ...73, Mason *et al.* 1974, Hemminki 1975) but, it has remained unestablished, whether the ...ocesses investigated have been due to binding or transport of calcium.

In this study highly pure preparations of bovine outer segments, intact and lysed, were ...ed to differentiate between binding and transport of calcium. The intact and lysed pre-...rations accumulated equal amounts of calcium in the absence of ATP (Fig. 3 A). This is ...strong argument against the possible contribution of mitochondria to the Ca^{++} accumula-

...ABLE VII. Lipid composition of outer segments. Means of 2 to 4 determinations.

...holesterol, protein (/w)	0.044
...hospholipid, protein (w/w)	0.58
Phosphatidyl ethanolamine (%)	42.7
Phosphatidyl choline	36.6
Phosphatidyl serine	15.3
Phosphatidyl inositol	1.3
Sphingomyelin	1.2
Phosphatidic acid	0.8
Lysophosphatidyl choline	1.8
Lysophosphatidyl ethanolamine	0.3

tion observed. Only a small net accumulation was observed after 1 min indicating that calcium may readily be taken up even by intact outer segments. ATP did not influence the initial accumulation of calcium but showed stimulation at later time points (Fig. 3B). The extent of stimulation was small in lysed outer segments, but marked in intact outer segments. These data suggested that the initial phase of Ca^{++} accumulation could be largely attributed to binding of Ca^{++} while the delayed phase, induced by ATP reflected active transport. A calcium ionophore, A 23187 was used to confirm these observations.

The present data support those of Bownds *et al* (1971) that the ATP-stimulated accumulation of Ca^{++} may be due to a membrane transport mechanism. Although plasma membranes are generally impermeable to ATP it was shown in the present study that the outer segments may be loaded to some degree with added ATP (Table III). It is thus likely that the membrane ATP site of outer segments is intracellular in analogy to other cell types. Yet we have so far been unable to demonstrate directly entrance of radioactive ATP into outer segments (Hemminki, unpublished) which may indicate that the effective concentrations of ATP are low in outer segments.

Binding but not transport of Ca^{+} to outer segments appeared to be regulated by light (Table IV). 20 to 40% more Ca^{++} was bound in the dark. Light dependence of Ca^{++} accumulation has also been reported elsewhere. Mason *et al* (1974) described, using sonicated vesicles of outer segments, that higher amounts of Ca^{+} were transported into outer segments in light than in the dark. In the present study no light dependent Ca^{++} transport was observed but the difference of the preparations must be recognized

Differential binding of Ca^{++} to bleached and unbleached outer segments was also demonstrated by a larger retention of Ca^{++} by unbleached preparations of lysed outer segments (Table V) It may be very important that in intact outer segments no such light dependence was observed presumably due to trapping of Ca released by plasma membrane. This is in excellent agreement of the model of visual excitation proposed by Hagins (1972). This implies release of Ca^{++} from discs on absorption of photons and blockade of Na permeability at plasma membrane by Ca^{++}

The excellent technical assistance of Mrs. Kirsti Salmela is appreciated Electron microscopy was performed at the Electron Microscope Laboratory Helsinki. The study was supported by the Sigrid Jusélius Foundation and the National Research Council for Medical Sciences, Finland.

References

BOWNDS, D A. GORDON WALKER, A.-C. GAIDE HUGUENIN and W ROBINSON, Characterization and analysis of frog photoreceptor membranes. *J gen. Physiol.* 1971 *58* 225–237

CARSWELL, A. H. and B. C. PRESSMAN Kinetics of transport of divalent cations across sarcoplasmic reticulum vesicles induced by ionophores. *Biochem. biophys. Res Commun.* 1972 *49* 29? 298.

DAEMEN F J Vertebrate rod outer segment membranes. *Biochim. biophys. Acta* (Amst.) 1973 300 ?–288.

FOREMAN, J C., J L. MONGER and B. D COMPERTS, Calcium ionophores and movement of calcium ions following the physiological stimulus to a secretory process. *Nature* (Lond.) 1973 *245* 249–251

HAGINS, W A., The visual process, excitatory mechanisms in the primary receptor cells. *Ann. Rev. Biophys. Bioeng.* 1972 *1* 131 158.

HELLER, J T J. OSTWALD and D BOX The osmotic behavior of rod photoreceptor outer segment discs

...HOFFMANN, K. Composition and synthesis of plasma membranes and smooth endoplasmic reticulum in isolated cells of newborn rat brain. *Biochim. biophys. Acta* (Amst.) 1973, 298, 810–816.
...HOFFMANN, K., Calcium binding to brain plasma membranes. *Biochim. biophys. Acta* (Amst.) 1974, 363, ...-210.
...HOFFMANN, K. Light-induced decrease in calcium binding to isolated bovine photoreceptors. *Vision Res.* 1975, 15, 69–72.
...HOFFMANN, K. and O. SCOVANNING, Preparation of plasma membranes from isolated cells of newborn rat brain. *Biochim. biophys. Acta* (Amst.) 1973, 298, 75–83.
...SON, W. T., R. B. FAGER and P. W. ABRAHAMSON, Ion fluxes in disk membranes of retinal rod outer segments. *Nature* (Lond.) 1974, 247, 562–563.
...GERALD, A. H., W. H. MILLER and M. W. BITENSKY, Calcium binding to retinal rod disk membranes. *Biochim. biophys. Acta* (Amst.) 1972, 266, 57–71.
...SON, S. R. STERN and T. H. McGOVAN, A rapid, accurate method for the determination of total cholesterol in serum. *Anal. Chem.* 1953, 25, 813–814.
...ATERS, D. D., K. BENCH and B. J. BARNETT, The preservation of cellular structure and enzymatic activity by aldehyde fixation. *J. cell Biol.* 1963, 17, 18–58.
...ATA, T. Electrical activity of vertebrate photoreceptors. *Quart. Rev. Biophys.* 1970, 3, 179–222.
...HAM, S. and W. A. HAGINS, Control of dark current in vertebrate rods and cones. In *Biochemistry and Physiology of Visual Pigments* (Ed. H. Langer), Springer, Berlin, Heidelberg and New York, 1973, ...43–255.
...ON, M. and S. FUTTERMAN, Extraction, regeneration after bleaching, and ion-exchange chromatography of rhodopsin in Tween 80. *Arch. biochem. Biophys.* 1973, 157, 91–99.

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Reflexogenic Contraction of the Ileo-Cecal Sphincter in the Cat Following Small or Large Intestinal Distension

By

P. E. Pahlin and J. Kewenter

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Abstract

Pahlin, P. E. and J. Kewenter. *Reflexogenic contraction of the ileo-cecal sphincter i[n the] cat following small or large intestinal distension.* Acta physiol. scand. 1975. 95. 126

The effect of distension of a small or large intestinal loop on the ileo-cecal sphincter (ICS) in the c[at was] studied with a flow recording technique. Distension of a small or large intestinal loop, isolated [from] the adjacent intestine, but with its mesenteric vascular and nervous supply intact, elicited [a con]traction of the ICS concomitant with an inhibition of the adjacent large and small intestinal m[otility.] Vagal and pelvic nerve section did not affect the response to distension of the ICS nor exclusion [of the] adrenal glands from the circulation. The sphincter reflex could be entirely or almost entirely ab[olished] by cutting both the splanchnic and lumbar colonic nerves, but not one or the other. Spinal sec[tion] blocked the reflex response indicating a spinal reflex arc. The reflex persisted after atropine an[d] pranolol, while both guanethidine and phenoxybenzamine completely abolished the reflex contrac[tion of] the sphincter.

The present results indicate that the excitatory intestino-ileo-cecal sphincteric reflex is a spinal refl[ex with] the main afferent and/or efferent fibres located within the major splanchnic and lumbar colonic [nerves.] The excitatory motor response in the sphincter is adrenergic and mediated *via* α-receptors.

Distension of a segment of jejunum or ileum, isolated from the adjacent intestine bu[t with] its vascular and nervous supply intact, elicits an inhibition of the small intestine *via* t[he so-]called intestino-intestinal inhibitory reflex, which is a spinal reflex under supraspina[l con]trol (Johansson and Langston 1964, Johansson *et al.* 1965 and 1968). Similar disten[sion of] a segment of the small intestine induces an inhibition of the motility of the proximal [colon,] the so-called intestino-colonic inhibitory reflex (Hultén 1969). In contrast, a contr[action] was obtained in the ileo-cecal sphincter (ICS) upon distension of a loop of the small a[s well] as the large intestine (Hinrichsen and Ivy 1931, Chang and Hsu 1942). Chang an[d Hsu] (1942) found that splanchnic, but not vagal, section abolished the latter reflex. Hinr[ichsen] and Ivy (1931) on the other hand could only eliminate the reflex by cutting both the [vagal] and splanchnic nerves, not one or the other. They were unable to determine the re[lative] importance of an extrinsic or an intrinsic intramural nervous mechanism in the

trol of the sphincter. The excitatory response of the sphincter to intestinal distension ht, theoretically be either cholinergic, adrenergic or both as it has been shown that both nergic and cholinergic nerve stimulation elicits a contraction of the ICS (Pahlin and nter 1975). Neither can a non-adrenergic, non-cholinergic mechanism be excluded as as been shown that such a transmitter mechanism must be involved in the extrinsic ous control of the gastrointestinal motor activity (e.g. Martinson 1965, Hultén 1969).

n view of these partly conflicting results, it was thought of interest to investigate further reflexogenic effect of intestinal distension on the ICS with respect to the nervous path- s and peripheral transmittor mechanism.

Methods

adult cats of both sexes, deprived of food for 24 h but with free access to water were used. After in- tion with ether the anesthesia was continued with chloralose (50 mg/kg b.w. i. .). A tracheal cannula inserted. The blood pressure was measured via femoral artery.

ording of transsphincteric flow

abdomen was opened by midline incision. The ileum was divided about one cm proximally to the and crushing ligature was tied around the colonic wall 1–2 cm distally to the ICS. In this ay the -cecal region was isolated from the adjacent parts of the gut but th its mesenteric nervous and vascular ply intact. A tube was inserted in the aboral end of the divided ileum and fixed with ligature imme- ately above the ICS. The ileal motor activity could thereby not influence the flow through the sphincter. ysiological saline (38°C) was led from reservoir to the sphincter via the tube. Since the cross-sectional of the reservoir was large (370 cm²) the perfusion pressure could be kept almost constant by means of erculation of the saline. The inflow pressure was usually set at about 5 cm H_2O. The saline reaching the ximal colon through the sphincter was drained by funnel inserted with its wider end through an ision at the antimesenteric border of the cecum. The saline was led to Gaddum-recorder (Gaddum 9) and volume changes in the recorder reflected the flow through the sphincter. The changes were istered via piston-recorder on smoked drum.

cording of intestinal motility. Distension of intestinal loop

estinal loops from the jejunum, ileum (about 15 cm in length) and colon (10 cm) were prepared either record the motor activity or to be used for intestinal distension. The small intestinal loops were isolated m the adjacent gut by transection of the wall and the colonic loops by crushing ligatures. The mesenteric rvous and blood supply to the loops was intact. The proximal ends of the small intestinal loops were sed by ligatures and glass cannula was inserted into the distal end and fixed with ligature. In the lonic loop the cannula as inserted through an incision in the antimesenteric border and fixed with ethylene string suture. The loops used for recording intestinal motility were connected to adaptable reservoirs coupled to volume recorder. The systems were filled with normal saline at body temperature. — The loops ed for distension were coupled to reservoirs that could easily be adapted to different heights. These stems were also filled with normal saline at body temperature. Distension pressures from 30–100 cm H_2O were used. The interval between the distensions was usually 10 min or more.

inal anesthesia. Nervous stimulation

5 cats spinal anesthesia was used to study whether the intestino-ileo-cecal reflex is spinal reflex or ediated via prevertebral reflex arc. Through cervical laminectomy at C VI and C VII thin poly- hylene catheter was inserted intradurally about 2 cm in the caudal direction. 1.2 ml of the anesthetic as injected through the catheter. Artificial respiration had to be instituted by means of respiratory mp in 3 of the animals. The left major splanchnic nerve was divided in 4 of the cats subjected to spinal anesthesia. A silver coil electrode was mounted on the distal end of the cut splanchnic nerve for stimulation th square wave impulses by means of Grass stimulator (S4E) at supramaximal voltages and durations -12 V 3-5 ms). The length of the stimulus trains as 30–60

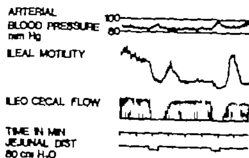


Fig. 1 Effect of jejunal distension on the arterial b. pressure, ileal motility and transphincteric flow. distension elicited a slight increase of the blood pre inhibition of the ileal motility and a contraction of sphincter. Adrenal glands ligated.

Intestinal distension was performed before and after cervical vagotomy in 9 cats. In 2 of these the p nerves were cut as well.

The 2 major splanchnic nerves were divided subdiaphragmatically in 9 animals. In 6 of these anim effect of intestinal distension on the transphincteric flow was also studied after the lumbar colonic n had been cut as well. In 3 cats the effect of intestinal distension was studied when only the lumbar col nerves had been cut.

Catecholamines from the adrenal glands are known to be a potent factor in the regulation of the inte motor activity (Kock 1959). In order to exclude the hormonal influence from the adrenals, the v of these glands were tied off. In 2 cats the ligature was performed d ring the experiment.

Drugs

The following drugs were administered intravenously: To block the "muscarinic" cholinergic rec atropine (Atropine sulphate, Merck) 1 mg/kg b.w.

To block the adrenergic α receptors, phenoxybenzamine (Dibenyline® Smith, Kline & French) 3 m b.w.

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Results

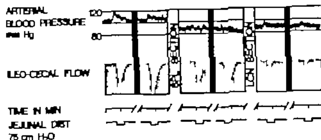
Distension of a jejunal (22 cats), ileal (6 cats) or proximal colonic loop (4 cats) reduced stopped the transphincteric flow in all cats. The sphincter contraction was obtained a distension pressure between 30 and 100 cm H O Concomitant with the sphincter contra tion there was an inhibition of tone and motor activity of the isolated large or small inte nal loops (Fig. 1). The results of the following experiments were all obtained by distens of a small intestinal loop

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No difference was observed in the sphincteric response to intestinal distension before a after the adrenal glands had been tied off from the circulation by ligatures (2 animals).

The intestino-ileo-cecal sphincteric reflex was unchanged after the vagal nerves had be cut in the neck and also when both the vagal and pelvic nerves were cut.

The reflexogenic sphincter contraction was unchanged or only reduced to a minor ext



. 2. Effect of splanchnic and lumbar colonic nerve (LCN) section on the sphincter contraction elicited jejunal distension. Note that splanchnic section did not affect the reflex contraction of ICS. When the lumbar colonic nerves were cut as well the ICS response to jejunal distension as almost completely ... Adrenal glands ligated.

...ter bilateral splanchnic nerve section. However when the lumbar colonic nerves were cut as well, the contraction was totally or almost totally abolished (Fig. 2). When the lumbar colonic nerves, on the other hand, was cut first, the reflex response could still be elicited (Fig. 3).

In order to investigate whether the intestino-ileo-cecal sphincteric reflex was mediated via the abdominal ganglia or was dependent upon nervous connection with the spinal cord, ... expts. were performed. The ganglionic sympathetic blockade was induced by means of spinal anesthesia. The results obtained in one of these experiments are illustrated in Fig. 4. At in the panel A, distension of an ileal loop elicited contraction of the ICS. Splanchnic nerve stimulation (*b*) at 8 Hz 6 V and 4 ms induced a contraction of the sphincter and an increase in blood pressure. Spinal anesthesia was then performed (between panel A and B in the figure). Ileal distension (*a* in panel B) no longer causes any contraction of the ICS. The effect of splanchnic nerve stimulation (*b*′ in panel B) remained unchanged, however. These results indicate that the reflex is spinal. Contraction of the ICS to intestinal distension (*a* in panel C) was again recorded after the spinal anesthesia had worn off. In 3 of the 5 animals spinal anesthesia blocked the reflexogenic contraction of the sphincter. In the remaining 2 animals the spinal anesthesia strongly reduced the reflex contraction but the response was not totally abolished, probably due to incomplete spinal anesthesia.

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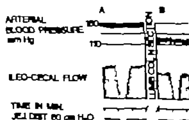


Fig. 3. Effect of jejunal distension on the transsphincter flow before and after section of the lumbar colonic nerves. Splanchnic nerves intact. Vagal and pelvic nerves cut. Both adrenal glands ligated.

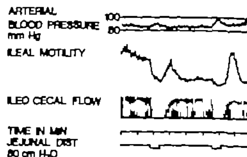


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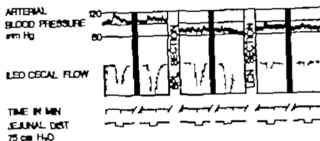
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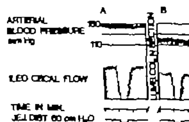


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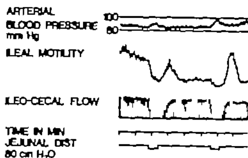


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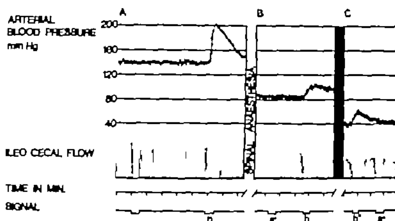


Fig. 4. Effect of spinal anesthesia on the ICS response to ileal distension and splanchnic nerve stimula-
a, a and a ileal distension. b, b and b splanchnic nerve stimulation (8 Hz, 6 V and 4 ms). Note that
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unchanged throughout the experiment. Both adrenal glands were ligated. The vagal nerves were cut at
neck.

Discussion

The present experiments have shown that distension of a small or large intestinal lo- elicits a contraction of the ICS concomitant with an intestinal inhibition. This effect is mediated *via* an extrinsic mechanism as the distended intestinal loop as well as the IC region was isolated from the adjacent gut, but with the mesenteric vascular and nerv- supply intact. A humoral adrenergic mechanism *via* adrenaline released from the adre- glands, could be excluded as the cause of the reflex contraction of the ICS as the refl- remained unchanged after the exclusion of the adrenal glands from the circulation.

Cervical vagotomy did not influence the intestino-ileo-cecal sphincteric reflex, which in accordance with the results of Chang and Hsu (1942). The reflex activation of the sphinc- could also be elicited in cats in which both the pelvic and vagal nerves had been cut. As t- reflex contraction could also be elicited after atropine it is concluded that the intestino-ile- cecal excitatory reflex is non-cholinergic and not mediated *via* the cranial or sacral par- sympathetic nervous system.

Guanethidine blocked the reflex, which makes an adrenergic peripheral transmittor mech- anism likely. The reflex contraction of the sphincter was also blocked by phenoxybenzami- but not by propranolol. It is therefore concluded that the sphincteric contraction elicit- by distension of an intestinal loop is mediated *via* an α adrenergic receptor mechanism. Th- is in accordance with an investigation of the adrenergic receptor mechanism in the IC- which showed that contraction of the sphincter elicited by adrenergic nerve stimulatio- was mediated *via* α-adrenergic receptors (Pahlin and Kewenter to be published).

The disappearance of the intestino-ileo-cecal sphincteric reflex after spinal anesthe- indicates that the reflex response of the ICS to intestinal distension is dependent upo- nervous connection with the spinal cord and cannot be elicited *via* decentralized abdomi- ganglia.

The reflex could be activated even after the major splanchnic nerves had been cut, althoug

...flex contraction of the ICS was decreased in some of the experiments. When the lumbar colonic nerves were cut as well, the reflex was totally or almost totally abolished. The effect of lumbar colonic nerve section on the sphincter reflex may suggest that the whole or almost the whole reflex is mediated *via* the lumbar colonic nerves. However when the lumbar colonic nerves were divided while the splanchnic nerves were intact the response was unchanged or only blocked to a minor extent. This suggests that both the splanchnic and the lumbar colonic nerves are involved in the mediation of the reflex contraction of the ICS during distension of a small intestinal loop. In some of the experiments a small reflex contraction of the ICS persisted even though the major splanchnic and lumbar colonic nerves were cut. This must indicate that the reflex can be at least partly mediated *via* nervous connections with the spinal cord outside the main sympathetic nerve trunks, *i.e.* the major splanchnic and lumbar colonic nerves.

Chang and Hsu (1942) found that the reflexogenic response of the sphincter varied with the sphincter tone. Thus, distension induced an inhibition of the sphincter when the tone was high and *vice versa*. In the present experiments intestinal distension did not induce relaxation of the sphincter when the tone in the ICS had been increased by splanchnic nerve stimulation or distension of another intestinal loop. Instead, a further contraction of the sphincter was obtained. Thus, no inhibitory fibres to the sphincter could be demonstrated in the present study. However this does not exclude the existence of such fibres, which might possibly be activated under other experimental conditions.

The intestino-ileo-cecal reflex is with respect to the reflex limbs and nervous connection with the spinal cord very similar to the intestino-intestinal inhibitory reflex (Johansson and Langston 1964) and the results obtained by Hultén (1969) for the intestino-colonic inhibitory reflex. In fact, it seems reasonable to assume that these reflexes, which are elicited by intestinal distension, are induced simultaneously and mediated *via* similar reflex arcs. This is supported by the present experiments, in which the motor activity of the small and large intestine was recorded concomitant with the transsphincteric flow.

Distension of an intestinal loop has been shown to produce a rapid reflex inhibition of the stomach and the small and large intestines (Johansson and Langston 1964, Jansson and Martinson 1966, Hultén 1969) and of the sphincter of Oddi in dogs (Nakayama 1969). Similarly antral distension elicits a reflex inhibition of the corpus-fundus via an adrenergic spinal reflex (Abrahamsson 1974). Contrary to these reflexes, the intestino-ileo-cecal sphincteric reflex is an excitatory reflex. The functional significance of this excitatory reflex is unclear.

The results of the present study differ from the findings in man by Cohen *et al.* (1968) and in dogs by Kelley *et al.* (1966). These authors found that ileal balloon distension elicited a pressure fall in the sphincter while they obtained a rise in the intrasphincteric pressure with colonic balloon distension. Distension of the intestine above as well as below the ICS always elicited a contraction of the sphincter in the present experiments. Whether this difference is due to a species difference or some other factor remains to be investigated.

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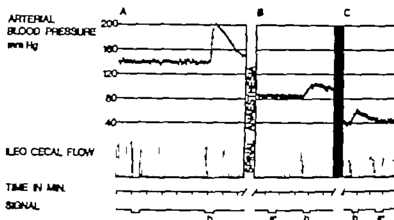


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HULTÉN, L., Extrinsic nervous control of colonic motility and blood flow. An experimental study in the cat. *Acta physiol. scand.* 1969. Suppl. 335.

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approaches were used to investigate to what extent various amines, both endogenous and exogenous to rat mast cells, taken up by the cells were stored in the granules: a) Compound 48/80 is known to release the endogenous amines HI and 5-HT from mast cells in a dose-dependent manner by a process involving exocytosis of amine-containing granules. Consequently the release curves for the two amines are almost identical (Moran, Uvnäs and Westerholm 1962). This being so other amines taken up by cell ought to be released parallel with the endogenous amines if they are localized to the granules.

b) Differential centrifugation of ultrasonically disintegrated mast cells yield a mixture of electron dense granules having an intact membrane, and swollen, less electron dense granules which are devoid of a limiting membrane (Anderson *et al* 1974). The granules surrounded by an intact membrane can retain their amines even when suspended in a cation-containing medium (*e.g.* isotonic NaCl). This means that amines taken up by the cells and stored in the granules can be demonstrated directly.

In the present *in vitro* study all the amines investigated were taken up by the cells and stored to a greater or lesser extent in the granules.

Methods

Isolation of mast cells

Mixed cell suspensions were obtained from the peritoneal and pleural cavities of male Sprague-Dawley rats weighing 350–450 g and the mast cells were isolated according to the method described by Thon and Uvnäs (1966), as modified by Uvnäs, Åborg and Bergendorff (1970). The method was recently published in detail by Anderson *et al.* (1974).

Isolation of granules

Isolated mast cells were sonicated (MSE, 100 Watt) in 0.34 M sucrose solution at 4 µm for 5 s and then subjected to differential centrifugation at 350 g for 10 min and 3 000 g for 20 min, all procedures being carried out at 4°C. The 3 000 g precipitate, containing a mixture of granules with an intact membrane and granules devoid of an intact membrane, was suspended in 0.15 M NaCl adjusted to pH 7 with Sörensen phosphate buffer and recentrifuged (3 000 g 20 min, 4°C).

The intensity and duration of sonication used was found to give the largest proportion (about 3%) of granules with an intact membrane (Anderson *et al.* 1974), Anderson and Uvnäs in press). Suspension of the mixed granule precipitate in 0.15 M NaCl at pH 7 releases all amines stored in the granules devoid of an intact membrane by cation exchange (Åborg, Novotný and Uvnäs 1967, Uvnäs, Åborg and Bergendorff 1970, Bergendorff and Uvnäs 1972, 1973). Thus the amines remaining in the granule mixture will be localized to granules with an intact membrane.

Charging of mast cells with amines

Isolated mast cells were pooled and suspended in buffered salt albumin solution (154 mM NaCl, mM KCl 0.9 mM $CaCl_2$ 10% Sörensen phosphate buffer pH 7 and 1 mg human serum albumin/ml) hereafter called the incubation medium.

Unlabelled amine and the corresponding ^{14}C-labelled amine were added to the mast cell suspension. The concentration of mast cells was usually 0.5–0.7 10^6 cells/ml, but was occasionally as low as 0.25 cells/ml. The level of radioactivity was usually 1 µCi/ml. In those experiments in which low amine concentrations were used the radioactivity was limited to 0.5 or 0.05 µCi/ml. The amine concentrations used in uptake studies varied between 10^{-4} and 3mM depending on the uptake of the amine (Fig. 1–4). In the release studies, solutions containing 0.1–0.15 mM of the amines were used, except for 5-HT and DA where concentrations of 0.005 mM and 0.02 mM respectively were used. At these concentrations the release of endogenous HI induced by compound 48/80 was not affected (Fig. 8 and 9).

The incubation of mast cells with amines was carried out in a shaking water-bath at 37°C for 90 min

...incubation, the cells were centrifuged down at 350 g for 10 min. The cells were washed in the incuba-...medium at room temperature until the radioactivity in the wash was close to background values (3 ...es, each of 10 ml).

...ent)

...mast cells. Cells loaded with the ^{14}C labelled amine under investigation were suspended in 10^{-4} M HCl ...heated in boiling water-bath for 5 min. Aliquots were taken for the assay of histamine and radio-...vity (for calculations see below) and, in few cases, for paper chromatography

...granules. Granules from mast cells loaded with the ^{14}C-labelled amine under investigation were pre-...d as described above. The precipitated granules were suspended in 10^{-4} M HCl and heated in boiling ...bath for 5 min in order to release the amines stored in the granules with an intact membrane. Aliquots ...taken for the assay of histamine, protein and radioactivity. For calculations see below

...fluorometric determination of unlabelled histamine. Histamine was assayed by the method of Shore *et al.* ...) by direct condensation with o-phthaldialdehyde, as described by Bergendorff and Uvnäs (1972).

...determination of ^{14}C-labelled amines. The radioactivity in aliquots of test samples and the ...sponding ^{14}C-labelled amine incubation solution was measured using Tri-carb® liquid scintillation ...trometer Model 3375 (Packard Instrument Co.), with Instagel® as scintillation fluid.

...alculations. At the beginning of each experiment, aliquots of the mast cell suspension were taken for the ...ry of endogenous histamine and for cell counting. From these results the histamine content per 10^6 ...was calculated. At the end of each experiment, aliquots were taken from each sample for the determina-...of radioactivity and endogenous histamine. Assuming that the Hi-content of intact mast cells does not ...nge during the incubation and washing procedures (checked in 3 expts.), the number of intact mast cells ...sent at the end of the experiment was calculated from the endogenous Hi-content at that time and the ...content per 10^6 cells at the start of the experiment.

...ata obtained from the above calculations enable direct comparison to be made between the amount ...amine taken up by cells and by membrane-bound granules.

...ease studies with compound 48/80

...Mast cells were incubated with the ^{14}C-labelled amine under investigation and washed free from external ...ioactivity as described above. The amine-loaded cells were exposed to different concentrations of com-...nd 48/80 (0.1 to 30 µg per ml), for 10 min at 37°C, and centrifuged down at 350 g for 10 min. Aliquots ...the supernatant and the sediment were taken for the determination of radioactivity and histamine.

...the paper chromatography

...Ascending paper chromatography was carried out using Whatman No. 1 paper and the following solvent ...tert-n-butanol/acetic acid/water (12 3 5). Reference chromatograms with ^{14}C-labelled and unlabelled ...amines were run in parallel. The chromatograms of the unlabelled amines were stained with ninhydrin. ...e radioactive chromatograms were cut into 1 cm strips which were cut into smaller pieces and placed in ...unting vials with 10 ml of Instagel® scintillation fluid and the radioactivity was measured as described ...ove.

...termination of ether water partition coefficient

...0 µl of ^{14}C-labelled amine solution (1 µCi) was added to 1 ml of 0.1 M sodium phosphate buffer, pH ...and 1 ml of ether saturated with this buffer. The two phases were thoroughly mixed and then allowed ...separate. Aliquots were taken from each phase and the ^{14}C-activity was recorded.

Materials

...stamine-(ring-2-^{14}C) dihydrochloride, sp. act. 59 mCi/mmole, tyramine-1-^{14}C hydrochloride, sp. act. 42 ...Ci/mmole, dopamine-^{14}C hydrochloride, sp. act. 59 mCi/mmole; DL-noradrenaline-(carbinol-^{14}C) ...L-bitartrate sp. act. 41.2 mCi/mmole, The Radiochemical Centre, Amersham, England. 5-Hydroxy-...ryptamine-2-^{14}C binoxalate, sp. act. 27.5 mCi/mmole, tryptamine-2-^{14}C bisuccinate, sp. act. 60 mCi/ ...mole, β-phenylethylamine-1-^{14}C hydrochloride, sp. act. 7 mCi/mmole; New England Nuclear Boston,

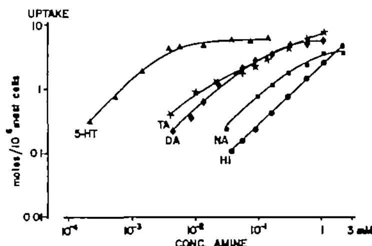


Fig. 1 Uptake by mast cells of 5-HT (▲—▲), DA (♦—♦), TA (✶—✶), NA (■—■) and HI (●—●).

Mass., USA. DL Ephedrine-1 ¹⁴C hydrochloride, sp. act. 20 mCi/mmole, D-amphetamine-7-¹⁴C sulphate, sp. act. 15.5 mCi/mmole Commissariat a l'Energie Atomique, Gif-Sur Yvette, France. Ficoll; AB Pharmacia, Uppsala, Sweden, human serum albumin (free from preservatives), AB Kabi, Stockholm, Sweden. All other substances were obtained from the usual commercial sources.

Abbreviations

Amph, amphetamine, DA dopamine Eph, ephedrine HI histamine, 5-HT 5-hydroxytryptamine, NA noradrenaline PhEA, β-phenylethylamine TA, tyramine TrpA, tryptamine.

Results

Uptake of amines into mast cells

All the amines investigated were taken up by isolated mast cells (Fig. 1 and 2). There were, however large differences between the uptake curves for the different amines. None of the amines produced any HI release in the concentrations studied Light microscopic observations did not reveal any morphological changes in the mast cells.

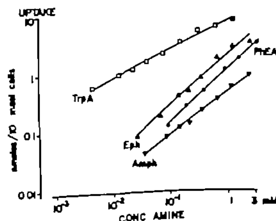
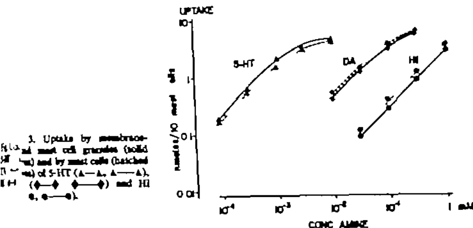


Fig. 2 Uptake by mast cells of TrpA (O—O), Eph (Δ—Δ), PhEA (O—O) and Amph (Δ—Δ)



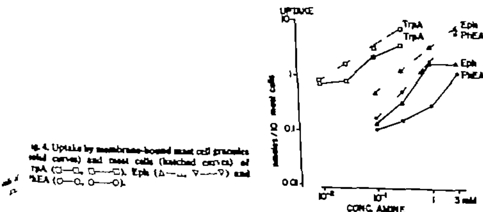
3. Uptake by membrane-...nd mast cell granules (solid ...es) and by mast cells (hatched ...es) of 5-HT (▲—▲, ▲----▲), ... (◆—◆ ◆----◆) and HI ●, ●----●).

...ribution of amines

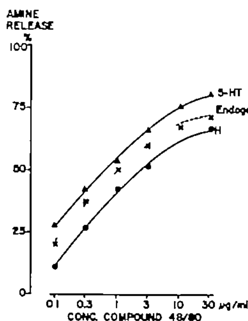
...take into mast cell granules. All amines studied were found to be localized to some ...ent in the granules (Fig. 3 and 4). The results indicate that HI, 5-HT and DA are mainly ...alized to the granules (Fig. 3), while a smaller proportion of the tryptamine (TrpA), ...edrine (Eph) and phenylethylamine (PhEA) (Fig. 4) taken up by the cells was found ...ere.

Amine release by compound 48/80. The compound 48/80-induced release of ¹⁴C HI and 5-HT from the mast cells occurred parallel to the release of endogenous HI (Fig. 5). ...ilar results were obtained for DA, NA and tyramine (TA) (Fig. 6). On the other hand, ...e release of PhEA, TrpA, Eph and amphetamine (Amph) (Fig. 7) did not run parallel ... the release of endogenous HI. The percentage release of the latter amines induced by ...compound 48/80 was considerably less than the HI release.

The HI-releasing effect of compound 48/80 was found to be influenced by the concentra-...on of DA used to load the mast cells with the amine (Fig. 8). Incubation in 0.02 mM



...g. 4. Uptake by membrane-bound mast cell granules ...olid curves) and mast cells (hatched curves) of ...rpA (□—□, □----□), Eph (△—△, ▽----▽) and ...hEA (○—○, ○----○).



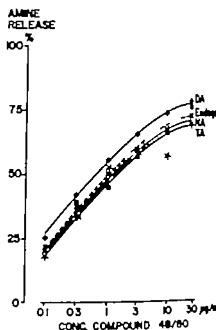
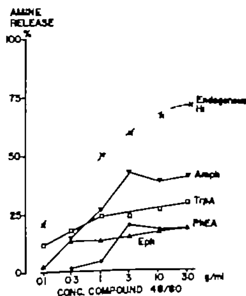


Fig. 5. Release of ^{14}C labelled 5-HT (▲—▲) and HI (●—●) and endogenous HI (——) induced by compound 48/80.

Fig. 6. Release of ^{14}C-labelled DA (◆—◆), NA (■—■) and TA (★—★) and endogenous HI(——) induced by compound 48/80.



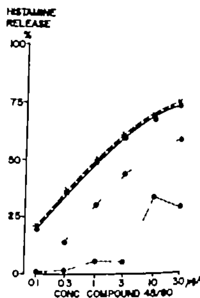


Fig. 7 Release of ^{14}C labelled Amph (▽—▽), TrpA (□—□), PhEA (○—○) and Eph (△—△) and endogenous HI (——) induced by compound 48/80.

Fig. 8. Compound 48/80-induced HI release from mast cells after incubation of the cells in 0.0... (⊕—⊕), 0.13 mM (⊕- -⊕) and 1.00 mM (⊕- -⊕) DA, control HI release (——).



9 Release of endogenous HI by compound 48/80 (1 µg/ml) after incubation of the mast cells in 5/HT TrpA (one concentration), Hi, TA, NA, Amph, Eph (two concentrations) and in DA (3 concentra-). S.D., standard deviation.

did not affect the compound 48/80-induced HI release. When higher concentrations of were used the HI-release was depressed (0 13 mM DA) or almost abolished (1 mM). The other amines produced analogous effects (Fig. 9). These findings were taken consideration when choosing the amine concentration used to load the cells in the dy of the release induced by compound 48/80.

...o paper chromatography

e chromatograms showed only one peak of radioactivity which coincided with the peak the radioactive reference and the ninhydrin-stained spot on the unlabelled reference omatogram. The radioactivity levels in the samples were too low to allow reliable cal- lations of the percentage radioactivity found in the peak to be made. The results owing only one peak for the amines chromatographed (HI, 5-HT PhEA, TA and DA) licate that little or no degradation of the amines took place during the experiments.

...her/water partition coefficient

e ether/water partition coefficients for the amines were compared with that of HI, which s found to be the most water-soluble of the amines studied (Table I).

Discussion

olated mast cell granules have been shown to store various cations (amines, sodium and alcium) rather unselectively by ionic binding (Bergendorff and Uvnäs 1973). HI and 5-HT e the only amines which occur naturally in rat mast cells. It was considered importan find out if amines which do not normally occur in rat mast cells could be stored ir ranules inside the cells.

When mast cells are exposed to compound 48/80 in a cation-containing medium, th ndogenous amines HI and 5-HT are simultaneously released from the granules after exo

TABLE I. Ether/water partition coefficients of various amines compared to that of histamine at pH
The compounds are listed in decreasing order of polarity

Amine	Ether/water partition coefficients relative to Hi at pH 7.4
Histamine	1
Dopamine	2
5-hydroxytryptamine	4
Noradrenaline	4
Tyramine	5
Phenylethylamine	28
Tryptamine	31
Ephedrine	47
Amphetamine	60

cytosis in exchange for cations in the extracellular medium. As the storage and rele processes for Hi and 5-HT are similar an amine which is taken up by the mast cells, tr ported into the granules and ionically bound there like Hi and 5-HT ought to be relea in parallel with the endogenous amines when the cells are exposed to compound 48/ Such parallel release was found for some of the amines investigated (Hi, 5-HT DA, N TA) which suggests that they are stored like Hi in the mast cell. On the other ha the release curves for PhEA TrpA Eph and Amph did not run parallel to that of end genous Hi. This finding may indicate that only a minor part of these amines is sto in the granules, as the release of these amines is less than the release of endogenous Hi the same concentration of compound 48/80 However it is still somewhat dependent the concentration of the releaser The major part of these amines may be located ex granularly More direct evidence that an amine is transported into the granules can obtained by studying the amine content of membrane-bound granules. Good agreem was found between the cellular and granular uptakes for 5-HT DA and Hi. No such cor lation was found for TrpA, PhEA and Eph, the calculated uptake into granules being than the cellular uptake. These results, like those of the release experiments, suggest th these amines are also localized to cell sites other than granules.

The cellular uptake of 5-HT is reported to take place by active transport, that of D and NA by facilitated diffusion and that of Hi by facilitated or passive diffusion (Ca and Haegermark 1966, Jansson 1970 Frisk Holmberg and Uvnäs 1972, Heisler and Uvn 1972). The use of different transport mechanisms by these amines is also reflected in th present results from the study of cellular uptake. Thus 5-HT is readily taken up by the ma cell and maximal uptake is obtained already at a concentration of 0.01 mM while to g the same Hi uptake a 100-fold higher concentration of Hi in the incubation medium is neede The uptake of Hi, like that of several other amines studied did not reach a maximu value in the range of concentrations used. These differences in uptake do not reflect th granular uptake. TrpA is more readily taken up by the cell than is Hi, but a smalle proportion is found in the granules. Hi and Eph have almost identical cellular uptak curves but a lower proportion of the Eph taken up by the cell is localized to the granul A correlation was found between the granular uptake and the polarity of the vario

nes (Table I). The more polar amines are mainly stored in the granules, while the less amines are stored there to a lesser extent. This could mean that the cellular uptake is erned by transport mechanisms in the cell membrane, while the granular uptake is main- lependent on the physico-chemical properties of the amines. Naturally selective intra- ular transport mechanisms may also exist.

n conclusion, the present results indicate that amines, both endogenous and exogenous at mast cells, taken up by the cell, are stored in the granules to some extent.

investigation was supported by The Swedish Medical Research Council Grant B74-14X-39-10C and rous Bergvalls Stiftelse. The skilful technical assistance of Margareta Wendling is gratefully acknowl- ed.

References

ang, C. H., J. Novotný and B. Uvnäs, Ionic binding of histamine in mast cell granules. Acta physiol. scand. 1967. 69. 552–567.

Bergendorff, P., P. Röhlich, S. A. Slorach and B. Uvnäs, Morphology and storage properties of rat mast cell granules isolated by different methods. Acta physiol. scand. 1974. 91. 145–153.

orsson, P. and B. Uvnäs, Selective localization of histamine to electron dense granules in antigen challenged sensitized rat mast cells and to similar granules isolated from sonicated mast cells. An electron microscopic autoradiographic study. Acta physiol. scand. 1975. 74. 63–73.

Bergendorff, A. and B. Uvnäs, Storage of 5-Hydroxytryptamine in rat mast cells. Evidence fo[r] ionic binding to carboxyl groups in a granule heparin-protein complex. Acta physiol. scand. 197[…] [8]6. 320–331.

Bergendorff, A. and B. Uvnäs, Storage properties of rat mast cell granules in vitro. Acta physiol. scand. 1973. 87. 213–222.

Lagunoff, U. G. Samuelsson and B. Uvnäs, Chemical composition of basophil granules from isolate[d] rat mast cells. Acta physiol. scand. 1971. 83. 362–372.

…son, M. and Ö. Hagermark, Uptake, storage and release of histamine by rat peritoneal mast cel[ls] in vitro. Acta physiol. scand. 1966. 68. 206–214.

…-Holmberg, M. and B. Uvnäs, The influence of chlorpromazine on the uptake of biogenic amines by rat mast cells in vitro. Acta physiol. scand. 1972. 86. 1–11.

…rane, A. V. and J. P. Green, Uptake of biogenic amines by mast cells of the rat. J. Physiol. (Lond.) 1964. 197. 263–271.

…, S. and B. Uvnäs, In vitro studies on the uptake of biogenic amines by rat mast cells. Acta physiol. scand. 1972. 86. 145–154.

…son, S.-E. Uptake of 5-hydroxytryptamine by rat peritoneal mast cells in vitro. Acta physiol. scand. 1970. 78. 484–492.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall, Protein measurement with the Fol[in] Phenol reagent. J. biol. Chem. 1951. 193. 265–275.

…lolan, N. C., B. Uvnäs and B. Westerholm, Release of 5-hydroxytryptamine and histamine from r[at] mast cells. Acta physiol. scand. 1962. 56. 26–41.

…hore, P. A., A. Burkhalter and V. H. Cohn, Jr. A method for the fluorometric assay of histamine [in] tissues. J. Pharmacol. exp. Ther. 1959. 127. 182–186.

…ney, I. L. and B. Uvnäs, Mode of storage of histamine in mast cells. Acta physiol. scand. 1966. 6[…] 455–470.

Uvnäs, B., C. H. Åborg and A. Bergendorff, Storage of histamine in mast cells. Evidence for an io[nic] binding of histamine to protein carboxyls in the granule heparin-protein complex. Acta physiol. scand. 1970. 78. Suppl. 336. 1–26.

Acta Physiol. Scand. 1975. 95. 142-144

From the Department of Physiology Karolinska Institutet, Stockholm, Swed

Noradrenaline Release Following Nerve Stimulation and its Modification by Prostaglandin E_2 in Human and Rabbit Oviduct

By

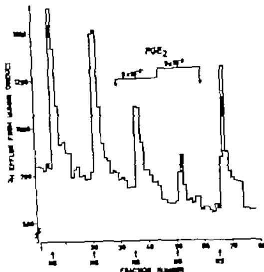
ATEF MOAWAD PER HEDQVIST and MARC BYGDEMAN

The oviduct muscular wall at the isthmus and especially at the ampullary isthmic jun- (AIJ) is heavily innervated with adrenergic fibres in all mammalian species studied, incl- man (Brundin 1965 Owman *et al.* 1967). The importance of the neuromuscular mechan in this region in the physiology of ovum transport has been the subject of many stu studies. Locking of the fertilized ovum at the AIJ for 48–72 h is said to be governed by adrenergic control mechanisms (Pauerstein 1974). Moreover prostaglandins (PGs) h been implicated in the modification of the physiological phenomena of this organ (Spi- 1974) The effects of PGs on the release of noradrenaline (NA) have been extensively st in other organs (*cf* Hedqvist 1974, Frame and Hedqvist 1975). The subject of this repo the study of the effects of nerve stimulation on the neurotransmitter release, its modifica by prostaglandins and the parallel effector responses in oviductal isthmus at the AIJ in t rabbit and the human.

The isthmic part of human and rabbit oviducts, 1½–2 cm in length, was preincubated for one h in Tyrode's solution containing 2.5 μCi/ml ^{3}H-( )-NA (sp.a. 5 Ci/mmol, N.E.N.). It was then mou in a 2 ml bath in a longitudinal orientation and was superfused with Tyrode at a rate of 1.5 ml The composition of the Tyrode was (conc. in mM) NaCl 136.7 KCl 2.7 $CaCl_2$ 1.8, $MgCl_2$ $NaHCO_3$ 11.9 NaHPO 0.4 glucose 5.5, ascorbic acid 0.1 and it was kept at 37°C and was ga with 5% CO_2 in O_2. The organ was transmurally stimulated by means of platinum wire electrode the wall of the bath and a Grass S4 stimulator delivering trains of biphasic pulses (5 Hz, 1 ms, sup maximal voltage) at 10–15 min intervals. Contractions were recorded by an isotonic transdu (Harvard 354) loaded with 0.5–1 0 g, and a Grass Model 5 polygraph. The superfusate was divi into one minute samples and the radioactivity was determined by counting 1 ml aliquots in a Pack liquid scintillation spectrometer using 10 ml Instagel as counting medium. Quenching was monito by internal standards. Prostaglandins were kindly supplied by Dr John Pike, Upjohn Co, Kalamaz U.S.A.

Transmural stimulation of superfused human and rabbit oviducts for 30–60 s perio (5 Hz, 1 ms, supramaximal voltage) consistently caused a marked and well reproduc increase in the release of tracer the bulk of which consisted of intact H NA. The stimulati parameters, as well as the finding that tetrodotoxin (0.2 μg/ml) and guanethidine (2 μg/ prevented the stimulation induced release of tracer and the resultant effector respo indicate activation of adrenergic nerve fibres.

Independent of hormonal condition, pregnancy estrus or progesterone dominance, t effector response to transmural nerve stimulation in the rabbit oviduct was consistently th of contraction In the human oviduct, however the effector response was variable, i.e. contraction, no response or even marked relaxation. Presumably and in contrast to t



...human oviduct previously loaded with ... Effect of PGE₂ on the outflow of ... response to transmural stimulation ...0 pulses at 5 Hz. Time in minutes ...on numbers.

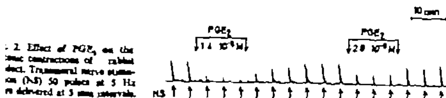
..., this is related to the hormonal condition of the subject, since it has already been ... that estrogen predominance enhances α-adrenergic receptor activity while pro-...one that of the β-receptors (Moawad and Kim 1974).

...ll hormonal conditions in the rabbit as well as in the human, PGE₂ in doses ranging 9 10^{-9} M to 9 10^{-6} M inhibited the release of ³H NA in response to transmural stimulation. The degree of inhibition was dose-dependent and the effect clearly re...le (Fig. 1).

...the rabbit oviduct of both estrogen and progesterone dominated animals PGE₂ caused ...tion of spontaneous motility. The contractions resulting from transmural nerve stimu-...s were also consistently reduced. The depression of both the spontaneous activity and ...erve stimulation responses were dose-dependent as well (Fig.).

...the human, in spite of the consistent inhibition of ³H-NA release, PGE₂ modified the ...uctal motility in various ways. While spontaneous motility when present, always was ...kedly depressed or abolished, contractions resulting from nerve stimulation were either ...tly depressed, not affected or even markedly enhanced. The one experiment where this ...r effect is well demonstrated is shown in Fig. 3. This particular tissue was obtained from ...bject in the immediate pre-ovulatory period. Presumably therefore, her estrogen pro-...tion was at a good level in conjunction with insignificant progesterone levels.

...hen the effector response to transmural nerve stimulation was that of depression, the ...able influence by PGE₂ should not be settled because of the almost complete cessation of



... 2. Effect of PGE₂ on the ...tonic contractions of rabbit ...duct. Transmural nerve stimu-...on (NS) 50 pulses at 5 Hz ...re delivered at 5 min intervals.

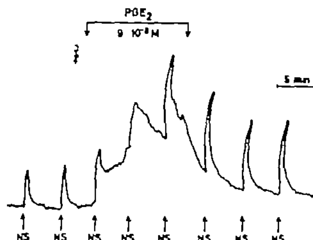


Fig. 3. Effect of PGE_2 on the motor contractions of a human oviduct in the follicular phase. Transmural stimulation (NS) 300 pulses at 5 Hz ... at 5 min intervals.

spontaneous activity in the presence of PGE_2. However in no case, was the relaxation ... nerve stimulation converted into one of contraction.

Comment

The isthmic region of human and rabbit oviduct is heavily innervated with adrenergic neurons. We have shown in these experiments that nerve stimulation results in increased ... release in both species. The invariable inhibition of this NA release by the addition of ... doses of PGE_2 is similar to results obtained from other adrenergically innervated ... (*cf* Hedqvist 1974 Frame and Hedqvist 1975).

In the rabbit oviduct there was a parallelism between restriction of transmitter release ... inhibition of effector response. The finding that nerve stimulation induced contraction ... the human oviduct sometimes were enhanced by PGE_2, in spite of depressed transmitter release, is unexpected. It points out the importance of species differences. Also, the result ... response in the human oviduct might well be related to the hormonal background of ... subject. Further detailed studies correlating the various responses to the hormone levels ... therefore essential.

This study was supported by grants from the Swedish Medical Research Council, project 04X-... A.M is on sabbatical leave from University of Chicago, supported by the Rockefeller Found...

References

BRUNDIN, J Distribution and function of adrenergic nerves in the rabbit Fallopian tube. *... physiol scand.* 1965. 66 Suppl. 259 1–57

FRAME, M. H and P HEDQVIST Evidence for prostaglandin mediated prejunctional control of ... vascular sympathetic tone. *Brit J Pharmacol.* 1975 54 189–196.

HEDQVIST P., Effect of prostaglandins and prostaglandin synthesis inhibitors on norepinephrine release from vascular tissue. In *Prostaglandin Synthetase Inhibitors*, eds. Robinson, H. J ... Vane, J R., pp. 303–309 Raven Press, New York. 1974

MOAWAD A. H and M KIM, Plasma sex steroid levels and the *in vitro* response of the isthmic part of human oviduct to catecholamines. *Gynec Invest* 1974 5 19–27

OWMAN, CH., E. ROSENGREN and N. O SJÖBERG Adrenergic innervation of the human female reproductive organs, a histochemical and chemical investigation *Obstet Gynecol.* 1967 30 ... 773

PAUERSTEIN, C. J *The Fallopian Tube A Reappraisal.* Lea and Febiger Philadelphia, 1974.

SPILMAN, C. H., Oviduct response to prostaglandins influence of estradiol and progesterone *Prostaglandins* 1974 7 465–469

Acta physiol. scand. 1975. 95. 145–152

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Cardiopulmonary Blood Volumes at Rest and during Muscular Exercise Measured by ^{113m}In Radiocardiography

By

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Received 18 March 1975

Abstract

Kuikka, J. T. Pyörälä, P. Lehtovirta and A. Rekonen. *Cardiopulmonary blood volumes at rest and during muscular exercise measured by ^{113m}In radiocardiography.* Acta physiol. scand. 1975. 95. 145–152.

Cardiac output, stroke volume, right heart and left heart blood volume, pulmonary blood volume and pulmonary mean transit time were measured at rest and during muscular exercise in 14 healthy subjects in the supine position. The method used was ^{113m}In radiocardiography measured precordially with a single detector. The results were analyzed with a minicomputer by a modified gamma function fitting method. The increase in cardiac output during exercise was 140 per cent ($p < 0.001$). The stroke volume increased per cent ($p < 0.05$) in subjects with heart rates < 160 beats/min, and decreased in subjects with heart rates > 160 beats/min. The right heart blood volume decreased from 115 ml/m² to 105 ml/m² (5 per cent; $p < 0.1$) and the left heart blood volume decreased from 120 ml/m² to 110 ml/m² (5 per cent, $p < 0.05$). The total heart blood volume thus decreased from 235 ml/m² to 215 ml/m² (5 per cent, $p < 0.05$). The pulmonary blood volume showed a corresponding increase from 250 ml/m² to 280 ml/m² (12 per cent, $p < 0.005$), and the cardiopulmonary blood volume did not markedly change (485 ml/m² and 495 ml/m²). Pulmonary mean transit time decreased from 5.0 to 2.4 (52 per cent, $p < 0.001$).

Key words: Radiocardiography, ^{113m}In, cardiac output, stroke volume, heart, cardiopulmonary blood volume, muscular exercise

Cardiac dynamic during exercise has attracted great interest. The variables measured have mainly been parameters of cardiac output, heart rate and blood pressure. Few studies have been concerned with the cardiopulmonary pools in man, because the methods giving information about central blood volumes are often inaccurate and rather cumbersome, requiring cardiac catheterization, and the results are difficult to analyze.

Radiocardiography has been used for decades for the determination of cardiac output. Its most obvious advantage is its versatility: it is atraumatic and requires only external monitoring. In addition, radiocardiography gives information about cardiopulmonary blood pools and flow times.

Henkanen (1971) and Iqbal and MacIntyre (1971) have presented a model for the analysis of radiocardiograms (RCG) with the analog computer. Digital computers are, however

more frequently available. In the present study we used a modified gamma functio fit method, first proposed by Thompson and co-workers (1964) and recently modified b (Kuikka *et al* 1974) for the digital minicomputer for the evaluation of the cardiopulm pools. At rest, radiocardiography gives the same results as other well-established techn (Donato *et al* 1962, Vernejoul *et al* 1964).

Vernejoul and co-workers (1964) have used radiocardiography to study changes in ca output and stroke volume at rest and during muscular exercise. Their results have establ the suitability of this technique in connection with exercise, too.

The purpose of the present work is to evaluate the basic results for the cardiopulmo parameters at rest and during heavy muscular exercise. The parameters measured are ca output, stroke volume, right heart, left heart and pulmonary blood volumes and pulmo mean time. All these parameters are measured simultaneously The digital computer is u for analysis of RCG curves. The results presented here form also the basis for correspo ing studies in connection with the use of contraceptive and anabolic steroids.

Material and Methods

Material. 14 healthy untrained test subjects were studied, 10 women and 4 men. Their mean age w years (range 19–32 years).

Procedure The subjects had fasted and had not smoked for 4 h before the study and had been on the measuring table for 30 min before the study Radiocardiography at rest was performed wi legs raised on the pedals of the ergometer 10 min after radiocardiography at rest, muscular exerci started. The load during exercise was 505 kpm/min (82 W) for women and 834 kpm/min (136 W) for Radiocardiography during exercise was done after 5 min bicycling (corresponding to a ork load of 2 kpm (24.6 kJ) for women and 4160 kpm (40.8 kJ) for men). At the time of radiocardiography the h rate had attained a stable level. Bicycling was continued to 12 min, when all blood samples for radioca graphy were taken. The blood pressure was measured with a sphygmomanometer immediately afte RCG curve recordi gs. The heart rate was followed co tinuously with a cardiotachometer (DV I, V met, Finland) connected to the chart recorder Measurements were made with the subject in the s position.

Radiocardiography Radiocardiography was perf rmed as described previously by Lehtovirta and workers (1972). A volume of 1 ml of ^{132}I (half life 100 min gamma ray energy 393 keV 100–400 was i jected i.v and the tracer dilution curv was registered precordially with a collimated scintil detector connected to the pulse height analyzer The RCG curv was recorded on a multichannel an (channel width 0.5 s or 0.25 s) nd the counts were registered by a printer In order to confirm a bolus delivered to the heart shoulder monitoring s important.

Blood samples were taken 7 and 10 min after the injection One sample taken during exercise be the radiocardiography was used for determining the background level of blood radioactivity.

Analysis of RCG curves. Cardiopulmonary parameters (cardiac output, right heart, pulmonary and heart blood volumes and pulmonary mean time) from the RCG curves were calculated by the mod gamma function f tting method (K ikka *et al.* 1974).

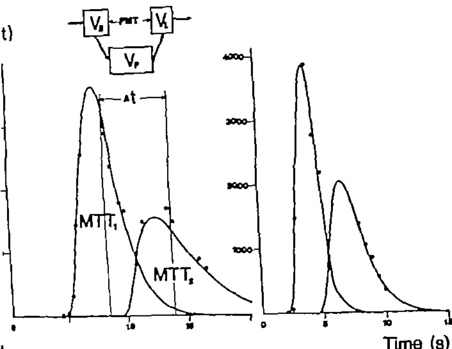
The sum of two gamma function integrands connected by the shortest transit time, t_p, between the r and left sides of the heart (atrium and ventricle combined) are fitted to the RCG curv

$$C_{RCG}(t) = K_1 t^{a_1} \quad {}^{t/b_1} + K_2 (t - t_p)^{a_2} \quad (t - t_p)/b_2$$

where a_i b_i = fitting parameters
K_i = scale factor

Index 1 refers to the right heart and index 2 to the left.

When cardiac output, CO, is determined the mean blood volume of each compartment, V_i, ca expressed with the aid of the shortest mean transit times, $\bar{t}$ as follows.



[illegible] 1. Radiocardiograms of [illegible] healthy female subject at rest and during muscular exercise. The measured [illegible] are marked with open circles. Digital computer analyses of radiocardiograms: CO = 6.88 l/min [illegible] 12.57 l/min, RHBV = 160 ml and 130 ml, LHBV = 150 ml and 140 ml, PBV = 520 ml and 460 ml; [illegible]T 4.6 and 2.2 s. The first peak of RCG curve shows the transit through the right heart and the [illegible] through the left

$$V = CO\ \bar{t}_i \quad \bar{t}_i = MTT - \sigma_i(m), \qquad (2)$$

[illegible] MTT = $b_i(a_i + 1)$ – mean transit time of tracer flow through the pool i, and

$\sigma_i(m) = b_i\sqrt{a_i + 1}$ – partial tracer distribution time of the pool (square root of variance).

[illegible] pulmonary blood volume, PBV which was modified slightly for this study is obtained from the equation

$$PBV = CO\ \bar{t}_p = CO\ \Delta t + PMV \qquad (3)$$

[illegible] $\bar{t}_p$ – pulmonary mean time

$$PMV = CO\ \sqrt{\sigma_2^2(m) - \sigma_1^2(m)} = \text{pulmonary mixing volume}$$

The cardiac output is determined in the conventional way from the RCG curve

$$CO = k\ \frac{h\ V}{A} \quad k = 0.81 \quad \text{equal}/T_{eff}, \qquad (4)$$

[illegible] A = $\int^{\infty} C_{RCG}(t)\ dt$ – area under the RCG curve (Eq. 1)

h – height of the equilibrium level

V – whole blood volume

k – correction factor

T_{eff} – effective half time of ^{113m}In

The fitting of the RCG curves was performed with NOVA 1220 minicomputer by the weighted least squares method (Bozzini and Clark 1970). Typical curves obtained in the analysis of the radiocardiograms at rest and during exercise are presented in Fig. 1.

TABLE I Individual results of cardiopulmonary parameters in 14 healthy subjects at rest and dur[ing mus]cular exercise.

Subject			Age (years)	BSA (m^2)		TBV (ml)	CO (l/min)	HR (b/min)	SV (ml/b)	RHBV (ml)	LHBV (ml)	HBV (ml)	PBV (ml)	CPBV (ml)
1	M. K	F	23	1.55	R	3 230	3.90	62	63	140	140	[illegible]80	40	520
					E	3 240	9.30	145	65	130	130	260	270	530
2.	I. M	F	22	1.57	R	4 030	4 43	65	68	180	180	360	290	650
					E	3 980	10.24	135	76	160	180	340	330	670
3	S. K.	F	[illegible]2	1.58	R	4 180	5.[illegible]4	75	70	220	230	450	330	780
					E	4 130	11 71	151	78	200	220	420	380	800
4	A. N.	F	19	1 63	R	4 490	3.98	63	63	160	210	370	400	770
					E	4 220	15.78	139	113	150	170	320	570	890
5	P K	F	21	1.66	R	3 950	5 43	75	72	170	150	320	450	770
					E	3 690	9 40	123	77	150	130	[illegible]80	500	7[illegible]0
6.	M. L.	F	2[illegible]	1 66	R	4 300	5.[illegible]4	53	99	180	230	410	650	1060
					E	4 1[illegible]0	14.90	133	112	210	210	420	660	1080
7	J V	M	32	1 76	R	5 110	5.50	69	80	190	220	410	520	930
					E	4 630	10.75	138	78	170	210	380	530	910
8.	P K	M	25	1.89	R	4 960	5.77	62	93	200	220	420	520	940
					E	4 770	15.12	147	103	200	220	420	570	990
9	A. T	M	27	1.89	R	5 330	6.[illegible]4	72	87	290	240	530	490	1020
					E	5 [illegible]40	14.02	142	99	270	240	510	510	1020
10	*P L.*	*M*	*32*	*2.06*	R	7 430	7.00	57	123	290	300	590	470	1060
					E	7 150	14 70	111	132	300	300	600	570	1170
11	*T P*	*F*	*21*	*1.50*	R	4 970	6.88	85	81	160	150	310	520	830
					E	4 860	1[illegible].57	165	76	130	140	270	460	730
12.	*P P*	*F*	*19*	*1.56*	R	3 670	5.84	70	83	230	70	500	580	1080
					E	3 310	10.43	160	66	180	220	400	360	760
13	*S. K*	*F*	*21*	*1.57*	R	3 370	8.50	81	105	150	150	300	570	870
					E	3 290	9 45	185	51	150	120	270	370	640
14	*L. H*	*F*	*19*	*1.57*	R	4 230	8.17	100	82	210	180	[illegible]90	470	860
					E	4 010	14 06	173	82	160	160	3[illegible]0	480	800

F = female, M = male, R = at rest, E during muscular exercise, BSA = body surface area, TB[V = total] blood volume, CO = cardiac output, HR = heart rate, SV = stroke volume, RHBV = right heart [blood] volume, LHBV = left heart blood volume, HBV heart blood volume, PBV = pulmonary blood v[olume], CPBV = cardiopulmonary blood volume, PMT pulmonary mean time

Results

The individual results for measured parameters at rest and during muscular exercis[e are] presented in Table I The average values for the most important parameters, cardiac i[ndex,] heart rate, stroke index, peripheral resistance, right heart, left heart and pulmonary b[lood] volume, both pulmonary mean time are shown in Fig. 2.

For normalization the volume values, the ratio of these volumes to the total blood vo[lume] was calculated in per cents. The mean right heart pulmonary and left heart blood vol[umes] at rest were (4.3 ± 0.6) %, (9 4 ± 2.6) % and (4 5 + 0 5) % and during exercise (4.3 + 0.[…] (11 0 ± 2.7) % and (4 4 ± 0.5) %. Correspondingly the mean cardiopulmonary blood vol[ume] was (18.2 ± 2.8) % and (19 7 ± 2.9) %.

In 5 subjects radiocardiography at rest was repeated after an interval of 15 min. […] individual values for cardiopulmonary parameters are found in Table II

In Table I the cases where the heart rate during exercise was equal to or greater than […] beats/min are italic. When divided into 2 groups (1 heart rate < 160 beats/min […]

Fig. 2a

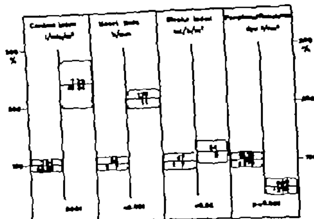
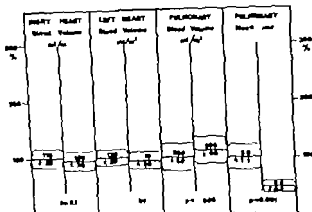


Fig. 2b



2. Mean values and standard errors of cardiopulmonary parameters in 10 healthy subjects (group 1, see text) at rest and during muscular exercise. The scale refers to the change of cardiopulmonary parameters in per cent.

...art rate 160 beats/min) the mean change in the stroke volumes between rest and exercise in group 1 is 15% and in group 2 22%.

The mean arterial pressure (MAP) was (98 ± 5) mm Hg at rest and (121 ± 7) mm Hg during muscular exercise. This increase was statistically highly significant ($p < 0.001$). The corresponding systolic pressure was (123 ± 7) mmHg and (162 ± 4) mm Hg ($p < 0.001$ for difference) and diastolic pressure (78 ± 3) mm Hg and (90 ± 14) mm Hg (p 0.05 for difference).

Discussion

The cardiac index and stroke index at rest are within the limits presented by many others (Donato *et al.* 196., Levinson, Pacifico and Frank 1966, Vernejoul *et al.* 1964). The rest values for cardiopulmonary blood volumes agree well with those presented by Peräsalo and Heiskanen (1971) and Iqbal and MacIntyre (1971).

During muscular exercise the mean increase in cardiac output was 140% in the group 1. This increase is of the same order of magnitude as found by Bevegård, Freyschuss and Strandell (1966) with a corresponding exercise load. Vernejoul and co-workers (1964) used

TABLE II Cardiopulmonary parameters of duplicate determinations in 5 healthy female subjects

Subject		HR (b/min)	CO (l/min)	RHBV (ml)	LHBV (ml)	PBV (ml)	PMV (ml)	PMT (s)
M	I	61	4.28	190	180	290	70	4.1
	II	65	4 62	170	190	280	70	3.6
K V	I	82	5 01	170	180	250	100	3.0
	II	85	4.95	190	170	180	60	2.2
S. P	I	52	5.28	250	220	330	60	3.8
	II	50	5.54	240	210	410	130	4 6
R. K.	I	79	7.26	290	250	670	210	3.3
	II	76	7.87	280	250	550	210	4.2
A. S.	I	96	7 35	200	170	400	120	3.3
	II	96	8.16	200	190	430	100	3.2
Mean	I	74	5 92	220	200	390	110	3.9
	II	73	6.22	220	200	380	110	3.6

I = first determination, II = second determination, HR = heart rate, CO = cardiac output, RHBV = r heart blood volume, LHBV = left heart blood volume, PBV = pulmonary blood olume, PMV pulm mixing volume, PMT = pulmonary mean time

lighter exercise (18 kJ) which is reflected in the smaller increase of heart rate (mean fro to 92 beats/min). The increase in cardiac output was correspondingly only 40%.

There is commonly held to be a 10-20% increase in the stroke volume during exe in untrained subjects (Bevegård Freyschuss and Strandell 1966, Vernejoul *et al.* 196 the group 1 (heart rate < 160 beats/min) the mean increase (15%) is within the tradit limits. The results in group 2 indicate that when the heart rate exceeds a certain limit 160 beats/min) the stroke volume begins to decrease, as reported for instance by Fa and co-workers (1971).

There was a mean decrease of 5 per cent in the right heart and in the left heart blo volume. The total heart blood volume thus decreased (5%), a finding which is in line w the reports that radiographic heart volume decreases slightly during exercise (Marshall Shepherd 1968) The decrease of the heart blood volume is assumed to occur in bo ventricles and these ventricular reductions are inversely related to ejection fractions.

The pulmonary blood volume increased 12 per cent (30 ml/m²) in group 1 Loepke co-workers (1971) report similar increase of pulmonary blood volume during musc exercise. In their studies these authors injected 2 indicators simultaneously into pulmon artery and left atrium. 3 hemodynamically normal subjects were studied in all three, the was a significant increment in pulmonary blood volume in exercise (mean 64 ml/m²). subjects with heart rate > 160 beats/min during exercise had a mean decrease of 20 per c in pulmonary blood volume.

The decrease in pulmonary mean time averaged 52 per cent If pulmonary mean tim calculated in heart beats (pulmonary blood volume/stroke volume), there is no differe between the values at rest (5.3 ± 1 1 beats) and during exercise (5.3 ± 0 9 beats), sugges a constant proportionality between pulmonary blood volume and stroke volume. There also a highly significant correlation (r = +0 85 p < 0 001) between the change in str

...me and the change in pulmonary blood volume. Therefore it seems that the pulmonary ...olume plays a role in the regulation of stroke volume.

...e dispersive part of pulmonary blood volume (Eq. 3), mean pulmonary *mixing volume* was 80 ml/m² at rest and 60 ml/m² during exercise. This dispersion volume across ...ngs averaged 25 per cent less during exercise than at rest. Knopp and Bassingthwaighte ...) have found a similar decrease in dispersion with increased cardiac output in the mon... dogs. The lung capillaries have low compliance and it is quite likely that some previously ...d pathways open when total blood flow is increased by increasing perfusion pressure ...din, Permutt and Maseri 1968). This explanation is also compatible with our observa... of increased mixing volume at long pulmonary mean time.

...e increase in the blood pressures and heart rate, and the decrease in the peripheral ...tance (48%) and blood volume (5%) are all within the accepted physiological limits ...ndall and Shepherd 1968).

...he present work gives for the first time the most important cardiopulmonary flow and vol... parameters measured simultaneously both at rest and during muscular exercise. A nonin...ve radioisotope method is used. The experimental verification of the parameters by in...ve, *catheterization methods is not performed*, but comparison with earlier separate works ...ws a close agreement as here is presented. The investigation is easy, is harmless to ... subject, gives reliable results and takes only 1/2 h for the measurement and analysis. ...alues of repeating measurements *do not normally* deviate more than 15 per cent (Table ...). The method is thus suitable for the study of cardiopulmonary parameters in a large group ... subjects in different situations and it is also suitable for clinical use.

...s investigation was supported in part by Population Council.

References

...olad, S. U. Freyschuss and T. Strandell, Circulatory adaption to arm and leg exercise in supine and sitting position. *J. appl. Physiol.* 1964, *19*: 37–46.

...ong, F. S. Permutt and A. Maseri, Effect of pulmonary vascular pressures and blood flow on CO diffusing capacity (Abstract). *Fed. Proc.* 1968, *27*: 379.

...Pratt, L., C. Giuntini, M. L. Lewis, I. Durand, D. F. Rochester, R. M. Harvey and A. Cournand, Quantitative radiocardiography (I), (II), (III). *Circulation* 1962, *26*: 174–199.

...ulkner, J. A., D. E. Roberts, L. R. Elk and J. Conway, Cardiovascular responses to submaximum and maximum effort cycling and running. *J. appl. Physiol.* 1971, *30*: 457–461.

...mbanck, T. Analogue model for the analysis of radiocardiograms. *Cardiovascular Res.* 1971, *5*: 268–276.

...m, Y. and W. J. MacIntyre, Analytical approach to dynamic radioisotope recording. *J. Nucl. Med.* 1971, *12*: 793–799.

...nopp, T. J. and J. B. Bassingthwaighte, Effect of flow on transpulmonary transport functions. *J. appl. Physiol.* 1969, *27*: 36–43.

...rkka, J., P. Lehtovirta, E. Kuikka and A. Rekonen, Application of the modified gamma function for the calculation of cardiopulmonary blood pools in radiocardiography. *Phys. med. Biol.* 1974, *19*: 692–700.

...htovirta, P., J. Kuikka, A. Rekonen and T. Pyörälä, Haemodynamic alterations produced by changes in posture with special reference to the usefulness of isotope ¹¹³ᵐIn radiocardiography. *Ann. Clin. Res.* 1972, *4*: 285–295.

...atinson, O. L., A. D. Pacifico and M. J. Frank, Studies of cardiopulmonary blood volume. Measurement of total cardiopulmonary blood volume in normal human subjects at rest and during exercise. *Circulation* 1966, *23*: 347–356.

LUEPKER, R B, LIANDER, M KORSGREN and E. VARNAUSKAS, Pulmonary intravascular and [illegible] fluid volumes in exercising cardiac patients. *Circulation* 1971 44 626–637
MARSHALL, R. J and J T SHEPHERD In: *Cardiac function in health and disease* W B. Saunders [illegible] 1968, 31–65.
PERÄSALO, J and T HEIKKANEN, Right heart, pulmonary and left heart blood volumes [illegible] analogue computer analysis of radiocardiograms in normal subjects and patients with [illegible]. *Cardiovascular Res.* 1971 5 260–67
STARMER, C. F and D O CLARK, Computer computations of cardiac output using the gamma [illegible] *J appl. Physiol.* 1970. 28 219–220.
THOMPSON H K., C. F STARMER, R. E. WHALEN and H D. MCINTOSH, Indicator transit time [illegible] as a gamma variate. *Circulat Res.* 1964 14 502 515
DE VERNEJOUL, P B DELALOYE, V DI GREGORIO and C. KELLERSHOHN, Mesure du débit cardiaque [illegible] des volumes ventriculaires par radiocardiographie. *Rév. Franç Étud. clin. biol.* 1964, 9, 693–[illegible]

Acta physiol. scand. 1975. 95. 153–165

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Metabolic Characteristics of Fibre Types in Human Skeletal Muscle

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Abstract

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scle biopsy samples were obtained from healthy subjects in order to evaluate quantitative differences single fibres of substrate (glycogen and triglyceride) and ion concentrations (Na^+ and K^+) as well as yme activity levels (succinate-dehydrogenase, SDH, phosphofructokinase, PFK, 3-hydroxyacyl-CoA ydrogenase, HAD myosin ATPase) between human skeletal muscle fibre types. After freeze drying the muscle specimen fragments of single fibres were dissected out and stained for myofibrillar ATPase h preincubations at pH's of 10.3, 4.6, and 4.35 Type I ("red") and II A, B, and C ("white") fibres could be identified. Glycogen content was the same in different fibres, whereas triglyceride content was hest in Type I fibres (2–3 X Type II). No significant differences were observed for Na^+ and K^+ between re types. The activity for the enzymes studied were quite different in the fibre types (SDH and HAD pe I 1.5 Type II. PFK Type I ≈ 0.5 Type II, Myosin ATPase Type I ≈ 0.4 Type II). The subgroups Type II fibres are distinguished by differences in both SDH and PFK activities (SDH, Type II C > A > B -K, Type II B A C). It is concluded that contractile and metabolic characteristics of human skeletal are are very similar to many other species. One difference, however, appears to be that no Type II fibres ve an oxidative potential higher than Type I fibres.

nce the first description of difference in colour between fibres in mammalian skeletal uscles many studies ha e further differentiated and characterized fibres in skeletal muscle. o some extent investigation has now reached the molecular level. However these studies ave not always focused on the characterization of the specific features of different fibre ypes. This is especially true for studies on human skeletal muscle where it is very difficult o obtain pure samples containing only one fibre type.

Recently *Peter et al.* (1972) described selected biochemical characteristics of the fibres ound in skeletal muscles of guinea-pig and rat. They were able to differentiate three types of fibres, which they named *fast twitch oxidative (red, Type II), fast twitch* glycolytic (white, Type II) and slow twitch oxidative (intermediate, Type I) fibres. In most species some muscles or muscle bundles contain only one type of fibres thereby *making it relatively* easy to characterize biochemically each fibre type separately. In muscles where the fibre types are mixed as in man (Johnson *et al.* 1973) it is difficult to obtain quantitative

measurements of one fibre type. Such information is needed, however to enable quantitative biochemical characterization of human muscle fibre types, and to better understand their metabolism and degree of adaptability

The difficulties described above can be partially overcome by using human muscle samples which contain known percentages of Type I and Type II fibres extrapolating biochemical analyses of both fibre type to 100% (Taylor Essén and Saltin 1974). However a better approach to this problem is to dissect each fibre type and to base qualitative biochemical measurements on either single or pooled fibres.

Using this technique the two main objectives of this study are to describe in detail methods that can be used to quantify substrate and ion concentrations as well as enzyme activity levels in fibres of skeletal muscles in man, and to describe some biochemical characteristics of these fibre types.

Material and Methods

Material. Approximately 150 muscle samples were taken and analysed from 35 healthy males and 5 females. The subjects differed in their background of physical activity and were 20–39 years old.

Muscle sampling. The muscle samples (10–50 mg) were usually obtained by a needle biopsy technique (Bergström 1962) and in some few cases by a surgical procedure. Before the biopsy was performed subjects were informed about the nature of the procedure and, as all of them had participated in similar types of studies before, they were well aware of both immediate and late side effects. The samples were taken at rest from the lateral portion of the thigh as well as from the deltoid, gastrocnemius and other muscles.

Classification of fibres. Based on histochemical stain for myofibrillar ATPase at pH 9.4 (Padykula and Herman 1955) after preincubation at pH 10.3 and 4.3 as well as NADH-dehydrogenase, these muscles, as most other skeletal muscles in man contain two major fibre types (Gollnick et al. 1972).

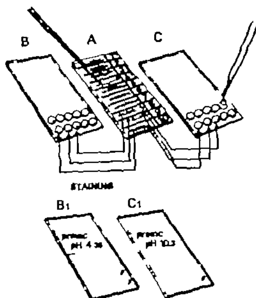
The fibres staining dark for myofibrillar ATPase at alkaline preincubation have been named Type II and the others Type I fibres (Engel 1964). The reverse staining pattern is obtained with the acid preincubation at pH 4.3, (Guth and Samaha 1969).

Preparation of fibres. Freeze dried material was used in order to obtain single muscle fibres. Freeze dried material absorbs O_2, N_2 and moisture when taken out of vacuum but reaches equilibrium in a few seconds (Lowry and Passonneau 1973). Absorption of approximately 5 % of the fibre weight can be assumed to occur at 20°C with less than 35 % humidity. Our dissection was therefore performed under such conditions. That no further change in fibre weight took place in our room was ensured by weighing the same fibre repeatedly during one day. The freeze dried material was placed under a dissection microscope (40×) and fragments of single muscle fibres were dissected out. The dissecting tools were constructed of wooden sticks with minute metal needles glued to one end.

The single fibres were then placed on a microscope slide, the ends cut off and placed in drops of water on another slide. After evaporation of the water the fibre ends were identified by staining for myofibrillar ATPase at pH 9.4 after preincubation at pH 10.3 for 15 min at 38 °C in 0.05 M glycine buffer 0.03 M $CaCl_2$ –0.05 M NaCl. The fibre ends were then stained either positively or negatively. Also the other fibre end was cut off from each fibre and stained after preincubation at pH 4.35 for 5 min at room temperature in 0.06 M NaAc–0.1 M KCl. This gave the opposite staining pattern also for single fibres.

After the fibres were classified the remaining portion of the fibre was weighed on a quartz fibre fish balance that had been calibrated with quinine hydrobromide. The weights of the fibres varied from ... to 5 µg. The above procedure is schematically illustrated in Fig. 1. In addition to the above staining procedure an acid preincubation at pH 4.6 was in some instances also included (Plate 3). As shown by Brooke and Kaiser (1970) this procedure allows a further separation of Type II fibres in subgroups A, B and C.

It is well documented that muscles in larynx, chewing muscles, and muscles around the eyes differ in their fibre characteristics.



1 The picture illustrates schematically staining procedure of individual muscle types. The fibres are divided in three (A) and the two small ends are each in a drop of water (B, C) that is allowed to evaporate. The result after staining myofibrillar ATPase after different pH C_1) is shown.

ensure as proper classification in subgroups as possible stains for NADH and -glycero-phosphate dehydrogenase were also used. These procedures called for extraordinary long fragments of muscle fibres and could then only be performed on small number of samples.

Biochemical methods. As in our earlier work the biochemical methods are based on NAD–NADP dependent enzymatic fluorometric (Farrand) assays.

Glycogen. A single fibre (0.5–5 µg) was placed in capillary tube and *8 µl of 1 M HCl* was added. *The* tube was then sealed and placed in an oven at 100°C for 2 h. From the hydrolyzed solution 2 µl was added 150 µl of reagent solution and analyzed for glucose residues (Essén and Henriksson 1974). The coefficient of variation determined on 12 divided fibres was 6.2 %.

Triglycerides. Pooled Type I or Type II fibres from the same muscle sample (30–100 µg) were homogenized in 0.5 ml of MeOH and 1 ml of $CHCl_3$. 1.5 ml of saline was added. After 24 hours the $CHCl_3$ phase was removed and evaporated under stream of N_2. 75 µl of 0.05 M E_4NOH in ethanol was added and the sealed tubes were heated at 60°C for 30 min in a water bath. 75 µl of 0.1 M HCl were then immediately added and the content mixed. Fatty acids were removed by shaking with 2 ml of hexane. The hexane layer was taken off and an aliquot of the hydrolysate was taken for glycerol determination according to Chernick (1969). Appropriate tripalmitin standards and blanks were treated in the same manner as the samples. Determination of the coefficient of variation on 6 pooled samples of the same type gave values as high as 35. As the error of the method of the analytical procedure only was 5 %, the large differences in values most likely reflects the *heterogeneous storage of lipids in the muscle fibres.*

Succinate dehydrogenase (SDH EC 1.3.99.1). From 5 to 10 µg muscle samples (3–15 fibres of similar type from one muscle sample) were used. The pooled fibres were incubated in 75 µl 0.3 M phosphate buffer (pH 7.7) with 0.05 BSA for 5 *min* at *room temperature.*

The sample was made 0.5 mM with regard to PMS (light sensitive), and 150 mM to Na-succinate and was incubated in water shake bath in the dark for 30 min at 38°C. Fumarate and malate were used as standards. The reaction was stopped with 25 µl 1 M NaOH and 50 µl bromthymol, mixed and centrifuged for 5 min.

A quantitative determination of the fumarate and malate produced was then made on the supernatant. 10 µl were added to 1 ml of 0.1 M hydrazine buffer (pH 9.0) containing 2.0 mM EDTA and 0.36 mM NAD. Blank fluorescence was read and the solution and 0.25 µg/ml of fumarase and 5 µg/ml of malate dehydrogenase added, and the reaction allowed to proceed for 2 h. The activity was expressed in mmol

fumarate or malate per kg dry weight per min. The coefficient of variation determined on 8 paired samples was 6.7 %.

Phosphofructokinase (PFK EC 2.7 1 11). A single fibre (0.5–2 μg) was added to 50 μl 0.04 M Imidazol buffer pH 7 6 and incubated for 2 min at room temperature.

The reaction was started with 1 ml reagent solution containing 0.04 M Imidazol buffer pH 7.6 $MgCl_2$, 1 mM ATP 5 mM ADP 5 mM P_i 0.2 mM 5-AMP 1 mM G-6-P 3 mM NH_4 0.01 mM NADH, 1 μg/ml phosphoglucoisomerase, 15 μg/ml aldolase, 3 μg/ml triosphosphatisomerase and 15 μg/ml glycerophosphatdehydrogenase. Fructose 1-6-diphosphate was used as standard. The test tube with the fibre was shaken every second minute. The activity was expressed mmol fructose-1-6-diphosphate produced per kg dry weight per min. The coefficient of variation for PFK determined on pairs of samples was 6.5 %.

3-hydroxyacyl-CoA-dehydrogenase activity (HAD EC 1 1 1.35). Determinations were made on the muscle sample homogenized in 0.3 M potassium phosphate buffer (pH 7.0).

The homogenate was added to a reagent solution containing 0.05 M Imidazol buffer pH 7.0, BSA, 0.1 mM EDTA, 0 05 mM NADH and 0.02 mM acetoacetyl-CoA. NADH was used as a standard with the blank being handled in the same way as the sample but without acetoacetyl-CoA. The activity was expressed as the maximal initial rate, mmol 3-hydroxyacetoacetyl-CoA produced per kg wet weight per min.

Myosin ATPase Muscle samples weighing 5–15 mg were homogenized in 5 ml of 0.15 M KCl–Histidine–0.005 M EDTA (pH 7.0). After centrifugation at 1500 g for 20 min the supernatant was discarded. The precipitate was washed in 0.15 M KCl–0.01 M Histidine (pH 7 0) and then dissolved in 6.5 ml of KI–6 mM $Na_2S_2O_3$ (pH 7.2). The sample was then centrifuged at 100 000 g for 30 min to remove ... and muscle residue. The supernatant was dialysed with distilled water overnight. The myosin was ... from the dialysis tube by centrifugation and resuspended in 0.5 M KCl pH 7.5. All the preparative procedures were performed at 4°C. Ca^{++} activated ATPase was assayed according to Sréter et al. (19..) final volume of 1 ml containing 0.05 M TRIS, 0.5 M KCl, 1 mM EDTA and 5 mM ATP. Incubation was stopped after 10 min at 25°C by adding 0.5 ml of 10 % perchloric acid. The liberated P_i was measured according to Rockstein and Herron (1951). Protein was measured as described by Lowry (1951). The activity was expressed as μmol P_i per mg per min. The fibre composition of the sample was determined on a ... which was histochemically stained for myofibrillar ATPase after preincubation both at pH 10.3 and 4.3 Pooled fibres (150–200 μg) were treated i the same way as the homogenates except that the ... in which they were homogenized was ml and the incubation time was 15 min.

K⁺ and Na⁺ concentrations were determined on single fibres (0.5–3 μg) with an integrating flame photometer (Nilham AB, Stockholm, Sweden). This apparatus was sufficiently sensitive to detect Na ... concentrations of 10^{-14} mol, thus allowing fibre size as small as 0.5 μg to be used. The accuracy of the method was within ±4 %.

0.5 μl of H_2O was used to attach the fibre to the platinum-iridium loop. Appropriate standards containing both Na^+ and K^+ were used and 0.5 μl of the solution was placed on the loop. The loop must be dry before it is placed in the flame, which was ensured by the use of radiation heat for at least 1/2 minute. ... revealed that neither of the ions nor proteins interfered with the determinations.

Results

Table I summarizes the observations made on fibres from human muscle which stained positively or negatively for myofibrillar ATPase after alkaline preincubation. It ... as if the difference in staining pattern corresponds to a real difference in the activity of myosin ATPase and the extrapolated values from the linear equation $y = 0.16 + ...$ (y = myosin ATPase x = fibre composition) comes out to be 0.16 and 0.48 mmol ... mg myosin per min for Type I and Type II fibres, respectively (Taylor Essén and ... 1974). These findings are further substantiated by determinations on pooled samples of fibres of the major types resulting in 2.2–2.5 times higher ATPase activity levels in Type II than in the Type I fibres.

TABLE I. Mean values ± S.D. and range for certain biochemical characteristics of skeletal muscle in [illegible] number of subjects).

[illegible]	Myofibrillar ATPase stain preincubated at pH 10.3	
	Positive (type II)	Negative (type I)
[illegible] mmol min⁻¹ mg⁻¹ [illegible] n = 2	0.48	0.16
Glycogen[b] mmol kg⁻¹ dw [illegible], 617 fibres	359 ± 92 (115–749)	355 ± 140 ([illegible])
Triglycerides[c] mmol kg⁻¹ dw [illegible] 4, 28 samples	74 ± 46 (21–164)	207 ± 86 (49–328)
Phosphofructokinase[b] µmol min⁻¹ kg⁻¹ dw [illegible] 12, 101 fibres	49.4 ± 7.3 (17.3–99.6)	25.8 ± 6.9 (8.0–61.3)
Succinate dehydrogenase[c] µmol min⁻¹ kg⁻¹ dw [illegible] 16, 52 samples	19.3 ± 7.6 (6.7–39.5)	29.6 ± 7.2 (22.7–50.6)
[illegible] mmol kg⁻¹ dw [illegible] 2, 400 fibres	632 ± 18 (598–702)	617 ± 21 (592–690)
[illegible] mmol kg⁻¹ dw [illegible] 2, 400 fibres	102 ± 7 (89–122)	98 ± 6 (86–115)

[a] Extrapolated values from linear regression (see text)
[b] Individual fibres.
[c] Pooled fibres.

The mean content of glycogen was 355 and 359 mmol per kg in the Type I and II fibres, respectively. The rather wide variation observed around these mean values is partly a [illegible]nction of different resting glycogen levels in the muscle studied. However, in most cases [illegible]5–90 [illegible] of the fibres in one muscle fell within 50 mmol per kg from the mean value. The [illegible]ntensity of the PAS stain varies somewhat but at near or above normal glycogen content [illegible] the muscle, the histochemical stain for glycogen content does not appear to be sensitive [illegible]nough (Plate 1 and Fig. 2).

The mean triglyceride concentration is almost 3 times higher in Type I as compared to [illegible] II fibres (207 vs. 74 mmol per kg d.w.). A large scatter around the mean value is also found for this substrate. This can probably be attributed to differential distribution of fat in the muscle samples. Thus a t-test on paired determinations, i.e. pooled II and I fibres from the same muscle sample, showed a highly significant difference (Fig. 2). The histochemical picture also indicates such a difference (Plate 1).

The only glycolytic enzyme studied, PFK, also demonstrated a significant difference

fumarate or malate per kg dry weight per min. The coefficient of variation determined on 1 paired samples was 6.7%.

Phosphofructokinase (PFK EC 2.7.1.11). A single fibre (0.5–2 μg) was added to 50 μl ... pH 7.6 and incubated for 2 min at room temperature.

The reaction was started with 1 ml reagent solution containing 0.04 M Imidazol buffer pH ... $MgCl_2$, 1 mM ATP, 5 mM ADP, 5 mM P_i, 0.2 mM 5-AMP, 1 mM G-6-P, 3 mM NH_4, ... 1 μg/ml phosphoglucoisomerase, 15 μg/ml aldolase, 3 μg/ml triosphosphatisomerase and ... glycerophosphatdehydrogenase. Fructose 1-6-diphosphate was used as standard. The test tube ... fibre was shaken every second minute. The activity was expressed mmol fructose-1-6-diphos... produced per kg dry weight per min. The coefficient of variation for PFK determined on pairs ... was 6.5 %.

3-hydroxyacyl-CoA-dehydrogenase activity (HAD EC 1.1.1.35). Determinations were made on ... muscle sample homogenized in 0.3 M potassium phosphate buffer (pH 7.0).

The homogenate was added to a reagent solution containing 0.05 M Imidazol buffer pH 7... BSA, 0.1 mM EDTA, 0.05 mM NADH and 0.02 mM acetoacetyl-CoA. NADH was used as ... with the blank being handled in the same way as the sample but without acetoacetyl-CoA. The ... was expressed as the maximal initial rate, mmol 3-hydroxyacetoacetyl-CoA produced per kg ... per min.

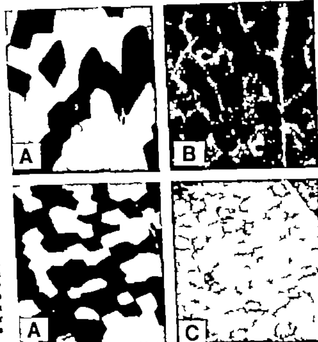
Myosin ATPase. Muscle samples weighing 5–15 mg were homogenized in 5 ml of 0.15 M KCl ... Histidine–0.005 M EDTA (pH 7.0). After centrifugation at 1500 g for 70 min the supernatant was ... The precipitate was washed in 0.15 M KCl–0.01 M Histidine (pH 7.0) and then dissolved in 0.5 ml ... KI-6 mM $Na_2S_2O_3$ (pH 7.2). The sample was then centrifuged at 100 000 g for 30 min to remove ... and muscle residue. The supernatant was dialysed with distilled water overnight. The myosin ... from the dialysis tube by centrifugation and resuspended in 0.5 M KCl pH 7.5. All the prepara... cedures were performed at 4°C. Ca^{++} activated ATPase was assayed according to Seiter et al. (19...) final volume of 1 ml containing 0.05 M TRIS, 0.5 M KCl, 1 mM EDTA and 5 mM ATP. Incubation ... stopped after 10 min at 25°C by adding 0.5 ml of 10 % perchloric acid. The liberated P_i was measured ... ing to Rockstein and Herron (1951). Protein was measured as described by Lowry (1951). The activ... expressed as μmol P_i per mg per min. The fibre composition of the sample was determined on ... which was histochemically stained for myofibrillar ATPase after preincubation both at pH 10.3 ... 4.3. Pooled fibres (150–200 μg) were treated in the same way as the homogenates except that the ... in which they were homogenized was 2 ml and the incubation time was 15 min.

K^+ and Na^+ concentrations were determined on single fibres (0.5–3 μg) with an integrating flame ... meter (Nilham AB, Stockholm, Sweden). This apparatus was sufficiently sensitive to detect Na^+ ... concentrations of 10^{-12} mol, thus allowing a fibre size as small as 0.5 μg to be used. The accuracy ... method was within ±4 %.

0.5 μl of H_2O was used to attach the fibre to the platinumiridium loop. Appropriate standards con... both Na^+ and K^+ were used and 0.5 μl of the solution was placed on the loop. The loop must be dry ... it is placed in the flame, which was ensured by the use of radiation heat for at least 1/2 minute. Pr... revealed that neither of the ions nor proteins interfered with the determinations.

Results

Table I summarizes the observations made on fibres from human muscle whic... positively or negatively for myofibrillar ATPase after alkaline preincubation. It ... as if the difference in staining pattern corresponds to a real difference in the activ... myosin ATPase and the extrapolated values from the linear equation $y = 0.16 + ($... (y = myosin ATPase, x = fibre composition) comes out to be 0.16 and 0.48 mmol ... mg myosin per min for Type I and Type II fibres, respectively (Taylor, Essén and ... 1974). These findings are further substantiated by determinations on pooled sam... fibres of the major types resulting in 2.2–2.5 times higher ATPase activity levels ... Type II than in the Type I fibres.



1. Photomicrographs (130) ... astus lateralis from one sub- ... illustrating myofibrillar ATP- ... staining after preincubation ... H 10.3 (A) and the PAS-stain ... glycogen (B) and OIL RED ... ain for neutral fat (C). Notice ... al amount of glycogen in both ... types but that the stain for ... tral fat is more intense in type ... ores.

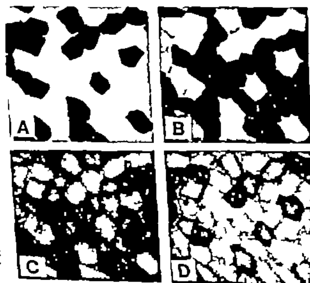


Plate 2. Photomicrographs (130) ... of vastus lateralis from one sub- ... ect illustrating myofibrillar ATP- ... ase staining after preincubation ... at pH 10.3 (A) and pH 4.35 (B), ... NADH-dehydrogenase (C) and ... α-glycerophosphatedehydrogenase ... (D). Notice the opposite staining ... pattern ... comparing micro- ... graph A and B.

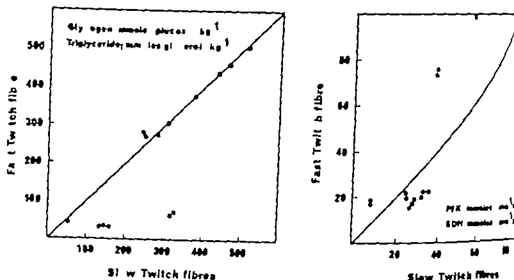


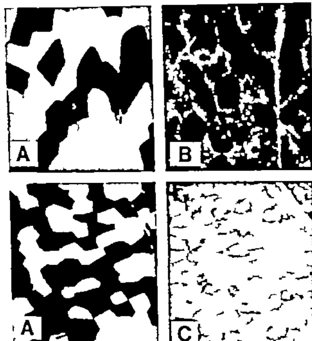
Fig. 2. The diagrams represent triglyceride and glycogen concentrations and the activity of SDH and PFK in type I (slow twitch) and type II (fast twitch) fibres from different muscle samples and subjects. One point shows the concentration of substrate or activity in type I (x-axis) and type II (y-axis) in the sample.

between the fibre types. In all cases where a paired comparison between fibres from the same muscle could be made, the highest activity was found in the Type II fibres (F... The stain for α-glycerophosphate-dehydrogenase is often used as a marker for glycolytic potential and from Plate 2 it can be observed that there is a definite difference between Type I and II fibres and minor variations exist between fibres of the same type.

Fibres positively stained for myofibrillar ATPase (alkaline preincubation) have the l... staining intensity for NADH-dehydrogenase. As this latter stain is an indicator of the oxidative potential of the fibre, there is a good agreement between the histochemical p... and the quantitative determinations of SDH (Plate 2 and Fig. 2). Muscle samples fr... subjects were used to study the SDH activity in Type II A, B and C fibres as well as Type I fibres. Of these samples, three were large enough to stain not only for myofi... ATPase (3 different preincubations) but also for NADH and α-glycerophosphatedeh... genases. Moreover on these three samples the PFK-activity was also determined. It ap... that within one muscle the SDH-activity in Type II A fibres is lower than the activ... Type I fibres but higher than in Type II B (p 0.05 Table II). The SDH-activity in II C fibres is very close to the activity in Type I fibres, but these fibres are very few (of total number of fibres).

The PFK-activity differs between Type I and II fibres and when comparing the groups of Type II fibres, a somewhat higher PFK-activity appeared in II B fibres.

The activity for HAD was only evaluated on samples with a mixed fibre popul... (30–90% Type I fibres). The activity was approximately 2 times higher than the ... activity giving values between 10–20 mmol x (kg min) When relating the HAD ac... to fibre composition a similar pattern as for SDH was observed. This may indicat... same difference between fibres types for HAD as for SDH



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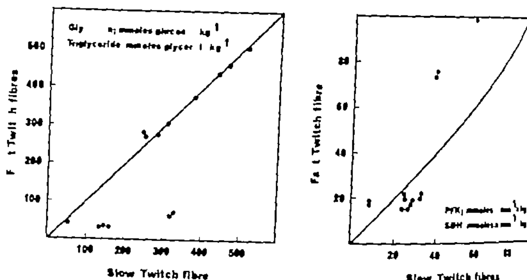


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The PFK-activity differs between Type I and II fibres and when comparing the subgroups of Type II fibres, a somewhat higher PFK-activity appeared in II B fibres.

The activity for HAD was only evaluated on samples with a mixed fibre population (30-90% Type I fibres). The activity was approximately . times higher than the SDH activity giving values between 10-20 mmol x (kg min) When relating the HAD activity to fibre composition a similar pattern as for SDH was observed. This may indicate the same difference between fibres types for HAD as for SDH

III. Comparison of determinations performed on muscle sample divided in two portions. One part was homogenized and the other part was freeze dried and dissected. The latter values are recalculated to wet weight based on water content of 76%.

	Homogenate	Type I	Type II	Type I+II (mean)
)cogen mol kg⁻¹ 17	58.5	54.6	58.8	56.7
K mol kg⁻¹ min⁻¹ 12	20.5	6.2	11.9	9.0
)H mol kg⁻¹ min⁻¹ 10	9.7	7.5	5.4	6.4

ys been obtained. The concern by Guth and Samaha (1969) that some fibres show a false ...ative stain for myofibrillar ATPase due to the staining of mitochondrial ATPase is most ...ely not a major problem in the present study. None of the subgroups of the Type II ...res have as high oxidative capacity as Type I fibres. Therefore, including the stain for ...yofibrillar ATPase with the preincubation at pH 4.35 and for NADH-diaphorase mini...zes a mistake in the classification (Fig. 1 and Plate 2).

To validate how representative the single fibre technique is, the following controls were ...rformed. The muscle fibre composition was determined in six muscle samples both on ...ss-sections of the samples and by counting the number of Type I and II fibres (~200 ...res) obtained from dissection. With these two procedures the mean difference in fibre ...mposition was 1.6%. The concentration of glycogen and the activity of PFK and SDH ...re determined on mixed muscles (homogenate) and individual fibres from the same ...scle specimen. For glycogen an almost complete agreement was found (Table III). The ...FK and SDH-activities, however, were consistently less when the determinations were ...rformed on freeze dried material. This may have the following explanation.

Freeze dried muscle tissues can be stored in vacuum at −20°C for several days without demonstrable change in the content of substrate or enzymes. After a day at room ...mperature in air substrate concentration is from a practical standpoint unaffected. How...er the activity of enzymes declines significantly within hours (Fig. 3). Thus when enzyme ...asurements are performed, the shortest possible time should elapse between the dis...ction and the assay. In the present study the assays were performed within 4–12 h, and PFK and SDH assay within 4–6 h. This then means that the values obtained for SDH ...tivity were reduced approximately 50% (Fig. 3). Taking this into account there is a ...sonable agreement also for the enzyme determinations.

At present we have no method to determine both wet and dry weight of the fibres, and ...alues, except for myosin ATPase, are therefore expressed in relation to the dry weight. ...s the portion of extracellular material around the fibre may vary slightly a determination ...f the true extra- and intracellular mass would be valuable. This is especially appropriate ...r the determinations of ion content.

TABLE II In the table is given the enzyme activities for succinatedehydrogenase (SDH) and phosphofructokinase (PFK) in different fibre types in human skeletal muscle (one subject). The measurements were performed on fragments of fibres and mean values of 3–5 fibres and the range are given. No correction for loss of enzyme activity has been made (cf Fig. 3 and Table III).

	Type I	Type II A	Type II B	Type II C
SDH	2.5	1 9	1 0	2.4
mmol × kg^{-1} × min $^{-1}$	(3 1–1.8)	(2.5–1.5)	(1.3–0.4)	(2.8–2.1)
PFK	3.9	5.6	6.9	5 5
mmol kg^{-1} × min	(4 9–2.1)	(6.4–5.0)	(8.3–4.5)	(5.9–5 0)

The potassium concentration averaged 617 and 634 mmol x kg for Type I and II (p < 0 05) respectively and the corresponding values for Na+ were 102 and 98 (p mmol x kg

Discussion

Separation of fibres in mixed muscles

The method presented in this study offers a possibility to separate fibres in mixed muscles in order to perform quantitative biochemical determinations not only on muscles of a specific fibre type, but also on individual fibres. In addition to the technical difficulties in determining the wet weight and the ratio between extra and intra-cellular volume of the fibres the main limitation in the method lies in the evaluation of sensitive biochemical assays on freeze dried material

Dissecting the fibres is a tedious procedure demanding skill and patience. It may take one hour to obtain 20–50 fibres. When the determinations are performed on single fibres it is of value to use as large fibres as possible. One important step is the classification of the fibres in Type I and II by staining for myofibrillar ATPase at pH 10 3 and 4.35. At times this proves difficult because a small number of fibres (<1–2%) do not stain either black or white, but more greyish It is of note that when a single fibre has been cut in several pieces (5–10) and all pieces are stained for myofibrillar ATPase an identical stain has

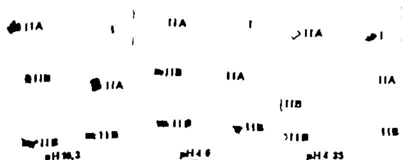


Plate 3. Photomicrographs (30) illustrating 3 fragments from six different fibres placed in the same order on three slides and stained for myofibrillar ATPase. In the left panel (slide) the preincubation performed at a pH of 10.3, in the middle at pH 4.6 and to the right at 4 35. A type I fibre stains white (10.3), white (4.6) and black (4.35). The staining pattern for type IIA fibre is black, white, white respectively, IIB fibre black, black and white.

LE IV Comparison between quantitative biochemical measurements performed on different fibre types found in guinea pig (Peter et al. (1972), rat (Baldwin et al. 1973), and muscles from man (Present study Table I). The ratio between Type II and I fibres (man) and between fast twitch glycolytic-oxidative (FTGO) and fast twitch glycolytic (FTG) and slow twitch oxidative (STO) fibres in guinea pig (rat) are given.

	Biochemical		
	Guinea pig/rat		Man
	FTGO/STO	FTG/STO	Type II/Type I
ntractile characteristics			
yosin ATPase	2.4	2.3	2.8
ubstrate			
lycogen	2.9	2.2	1.0
riglyceride[a]	1.2	0.8	0.4
colytic potential			
FK/α-glycerophosphate			
hydrogenase	2.7	2.7	1.9
idative potential			
DH	1.3	0.4	0.7

Rat muscle

erest to note our result on the SDH and PFK-activities of Type II (A and B) fibres. ey do indicate definite differences between the two subgroups of Type II fibres. Whether e difference is genetical or due to an adaptation cannot be concluded at present. Preminary studies using the disappearance of the PAS-stain from individual fibres have own that at high work intensities demanding 80–90% of maximal oxygen uptake, Type A fibres may lose PAS-stain at the same time as the Type I fibres (Andersen and Sjøgaard, personal communication). Type II B fibres in the same situation still stain intensely for glycogen. These results may then indicate different thresholds for activation of pe II A and B fibres, and the higher SDH-activity in the Type II A fibres is consequence of more frequent use of these fibres than the Type II B. The conclusion must therefore be that human skeletal muscle does not contain fast twitching fibre which has as gh or higher oxidative capacity as the slow twitching fibres of the same muscle. A FT bre with similar characteristics to the FTGO fibre in guinea pigs has been described in any different species including low forms of primates such as the Bush Babys (Gillespie, mpson and *Edgerton 1974). It would be of interest to investigate where in the phylogenetic* ea the FTGO fibre becomes less oxidative than the ST-fibre.

The different fibre types of guinea pig muscles have different membrane potentials as ell as potassium and sodium concentrations (Campion 1974). Also in human muscles fferent membrane potentials appear to exist between Type I and II fibres (Eberstein and oodgold 1968, Hanson 1974), as well as contraction times (Buchthal and Schmalbruch 970). We were unable to demonstrate significant differences in the K and Na concentration of Type I and II fibres. However we determined ion content on the whole fibre nd a valid estimation of intra- versus extracellular concentration of these ions in the fibre must be made before a conclusion on this particular point can be drawn.

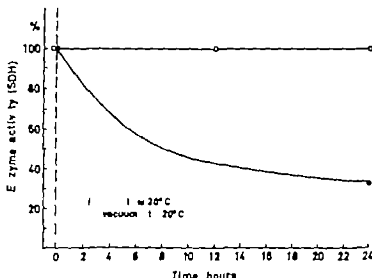


Fig. 3. The relationship bet SDH acti ity and time in air at 20°C and at at −20°C.

Muscle fibre types in man compared with other species

The question arises as to whether skeletal muscles of man contain more than two dist ly different fibre types. A comparison by Peter *et al* (1972) of some of the biochem characteristics quantitatively determined on "white" and red" vastus and also on sol muscles of guinea pigs may be useful The three muscles or portions of the muscles stud contain predominantly FT high glycolytic (FTG), FT high glycolytic–oxidative (FTO and ST high oxidative (STO) fibres (Gillespie, Simpson and Edgerton, 1970). The myo ATPase appears to be very similar for human Type I and guinea pig STO fibres, wher human Type II fibres have slightly higher ATPase activity than FTG and FTGO guinea pigs (Table IV) The glycogen content of human Type I fibres is considerably hig (5–6x) than that found in STO fibres of guinea pigs. In addition approximately 50 % m glycogen is found in the human Type II fibres. This results, therefore, in FTG/STO FTGO/STO ratios of 2–3 whereas Type II/I is 1 0 (Table IV). The lowest triglyc content is found in human Type II and rat FTG Thus the Type I fibre is well suppl both with glycogen and triglycerides. In fact skeletal muscle in man has 3–5 times m energy stored intracellularly than found in rats and guinea pigs.

No direct comparison between glycolytic enzymes can be made, but if the α-glyc phosphate dehydrogenase activity is used as an indicator of glycolytic potential in th guinea pig and PFK of that in human muscle fibres, fairly good agreement can be fou although minor quantitative differences may exist in ratios (Table IV).

When considering the oxidative enzymes it is quite obvious that the human Type (II A and B) and guinea pig FTG fibres exhibit the lowest activity whereas human Typ fibres are similar to the STO or FTGO fibres of guinea pigs. It may be argued that th very wide variation observed for both PFK and SDH activity in human Type I and fibres should favour the idea of more than two fibre types. It must then be remember that the measurements are performed on different functioning muscles of the body an also on muscles that are in different states of training. In this connection it is

IV Comparison between quantitative biochemical measurements performed on different fibre types found in guinea pig (Peter *et al.* (1972), rat (Reichmann *et al.* 1973), and muscles from man (Present study Table I). The ratio between Type II and I fibres (man) and between fast twitch glycolytic-oxidative (FTGO) and fast twitch glycolytic (FTG) and slow twitch oxidative (STO) fibres in guinea pig (rat) are given.

	Biochemical		
	Guinea pig/rat		Man
	FTGO/STO	FTG/STO	Type II/Type I
tractile characteristics			
osin ATPase	2.4	2.3	2.8
able			
ycogen	2.9	2.2	1.0
glyceride*	1.2	0.8	0.4
olytic potential			
K, α-glycerophosphate			
hydrogenase	2.7	2.7	1.9
idative potential			
DH	1.3	0.4	0.7

at muscle.

erest to note our result on the SDH and PFK-activities of Type II (A and B) fibres. ey do indicate definite differences between the two subgroups of Type II fibres. Whether difference is genetical or due to an adaptation cannot be concluded at present. Pre inary studies using the disappearance of the PAS-stain from individual fibres have own that at high work intensities demanding 80–90% of maximal oxygen uptake, Type A fibres may lose PAS-stain at the same time as the Type I fibres (Andersen and Sjö- ard, personal communication). Type II B fibres in the same situation still stain inten- ely for glycogen. These results may then indicate different thresholds for activation of pe II A and B fibres, and the higher SDH-activity in the Type II A fibres is a conse- ence of more frequent use of these fibres than the Type II B. The conclusion must there- re be that human skeletal muscle does not contain a fast twitching fibre which has as gh or higher oxidative capacity as the slow twitching fibres of the same muscle. A FT re with similar characteristics to the FTGO fibre in guinea pigs has been described in any different species including low forms of primates such as the Bush Babys (Gillespie, mpson and Edgerton 1974). It would be of interest to investigate where in the phylogenetic en the FTGO fibre becomes less oxidative than the ST-fibre.

The different fibre types of guinea pig muscles have different membrane potentials as ell as potassium and sodium concentrations (Campion 1974). Also in human muscles ifferent membrane potentials appear to exist between Type I and II fibres (Eberstein and oodgold 1968, Hanson 1974), as well as contraction times (Buchthal and Schmalbruch 970). We were unable to demonstrate significant differences in the K and Na concen- ration of Type I and II fibres. However we determined ion content on the whole fibre nd a valid estimation of intra- versus extracellular concentration of these ions in the fibre must be made before a conclusion on this particular point can be drawn.

Interconversion of fibre types

Experiments with electrical stimulation or cross-innervation in animals have shown t fibres can be converted one into the other (Bárány and Close 1971 Sréter et al. 197 Whether this occurs in human skeletal muscles is unknown. Different training proced appear to have little influence on muscle fibre composition when based on the stain myofibrillar ATPase (Gollnick *et al* 1973 Saltin *et al.* 1975) However this may not h for the subgroups of Type II A and B fibres as endurance training appears to increase percentage of II A on the expense of II B fibres (Jansson 1975 Andersen and Henrik personal communication) In fact, a conversion of Type I to Type II fibres may occur i in human skeletal muscle. Recently it has been found that patients who due to a le of the spinal cord are paretic in their lower extremities and develop spasticity have d to 100% Type II fibres in their thigh, gastrocnemius, and soleus muscles, whereas t deltoid muscles are mixed (Grimby personal communication)

Muscle fibre nomenclature

A long-standing controversy exists concerning the nomenclature to be employed for different muscle fibre types. Peter Barnard, Edgerton and colleagues (1972) have nomenclature for designating fibres which includes not only the fibres contractile but a metabolic characteristics. However such a nomenclature is based on the assumption o close correlation between enzyme activity levels of a fibre and the contractile characteris as well as the metabolism taking place in it, which remains to be proven for human mus Moreover the differences between human muscle fibre types may not be as distinct a most other species. Although all this is true, it may still be worthwhile to designate hu muscle fibres and fibre types by their basic characteristics in a manner similar to w has been done by Peter *et al* (1972) Type I II A and B fibres would then be called s twitch high oxidative low glycolytic, fast twitch oxidative–glycolytic and fast twitch h glycolytic low oxidative fibres, respectively In various species the absolute values for contractile and metabolic characteristics may be of completely different magnitude. Th the different terms when based on histochemical stains (slow–fast, low–high) are only re tive and cannot be used for quantitative comparisons of muscle fibre types in diffe species. Finally it may be worthwhile to emphasize that from functional and clinical sta points a subdivision of fibres beyond the two major groups of slow and fast twitch fibres may not so often be warranted

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References

BÁRÁNY M. and R. J CLOSE, The transformation of myosin in crossinnervated rat muscles. *J Phy (Lond.)* 1971 *213.* 455–474

BERGSTRÖM, J Muscle electrolytes in man. *Scand. J clin. Lab Invest* 1962. Suppl. 68.

BROOKE, M. H. and K. K. KAISER, Muscle f bre types How many and what kind? *Arch. of Neurol.* (Chi 1970. *23* 369–379

...TAL, F and H. SCHMALBRUCH, Contraction time and fibre types in intact human skeletal muscle. ...a physiol. scand. 1970. 79. 435–452.

...MAN, D. E., Resting membrane potential and ion distribution in fast and slow-twitch mammalian ...scle. J clin. Invest. 1974. 54 514–518.

...ARCK, E. E., Determination of glycerol and acylglycerol. Methods in Enzymology 1969 14 627–630.

...TAFF, A. and J. GOODGOLD, Slow and fast twitch fibres in human skeletal muscle. Amer J Physiol. ...1968. 215. 535–541.

...EL, W K., The uncertainty of histo- and cytochemical studies of skeletal muscle in investigation of neuromuscular disease. Neurology 1962. 12. 778–794.

...x, B. and J. HENRIKSSON, Glycogen content of individual muscle fibres in man. Acta physiol. scand. ...1974. 90. 645–647

...LNICK, C. A., D. R. SIMPSON and V R. EDGERTON, High glycogen content of red as opposed to white ...eletal muscle fibers of guinea pigs. J Histochem. Cytochem. 1970. 18. 552 558.

...LNICK, C A., D. R. SIMPSON and V R. EDGERTON, Motor unit recruitment as reflected by muscle fibre glycogen loss in ... prosimian (bushbaby) after running and jumping. J Neurol. Neurosurg Psychiat. 1974, 37 817–824.

...LNICK, P D., R. B. ARMSTRONG, C. W SAUBERT IV K. PIEHL and B. SALTIN, Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. J appl. Physiol. 1972. 33. 312 319

...LNICK, P D., R. B. ARMSTRONG, B. SALTIN, C. W SAUBERT IV W L. SEMBROWICH and R. E. SHEPHERD, Effect of training on enzyme activity and fiber composition of human skeletal muscle. J appl. Physiol. 1973 34. 107–111

...N, L. and F J. SAMAHA, Qualitative differences between actomyosin ATPase of slow and fast mammalian ...uscle. Exp. Neurol. 1969 25 138–152.

...SON, L., Effects of repetitive stimulation of membrane potentials and twitch in human and rat intercostal muscle fibres. Acta physiol. scand. 1974. 92. 238–248.

...SON, E., Type II A and B fibres in skeletal muscle of man. Acta physiol. scand. 1975. 94 In press.

...SON, M A J POLGAR, D. WEIGHTMAN, and D. APPLETON, Data on distribution of fibre types in thirty-six human muscles. An Autopsy Study J Neurol. Sci. 1973. 18 111 129.

...RY, O. H. and J. V PASSONNEAU, A flexible system of enzymatic analysis. Acad. Press, New York, 1973.

...RY O H N. J ROSEBROUGH, A. L. FARR and R. J. RANDALL, Protein measurement with the folin phenol reagent. J biol. Chem. 1951 193. 265–275.

...TTULA, H A. and E. HERMAN, The specificity of the histochemical method of adenosine triphosphatase. Histochem. Cytochem. 1955. 3 170–195.

...N, J. B R J. BARNARD, V R. EDGERTON, C. A. GILLESPIE and K. E. STEMPEL, Metabolic profiles of three fibre types of skeletal muscle in guinea pigs and rabbits. Biochemistry 1972. 11 2627–2633.

...MAN, J K M. BALDWIN and J. O HOLLOSZY Intramuscular triglyceride utilization by red, white and intermediate skeletal muscle and heart during exhausting exercise. Proc. Soc. exp. Biol. (N.Y) 1973 142. ...–431

...RITZEN, M and P W HARON, Colorimetric determinations of inorganic phosphate in microgram quantities Anal Chem 1951 23. 1500–1501

...TIN, B D. L. COSTILL, K. NAZAR, E. STEIN, E. JANSSON, B Essén and P D. GOLLNICK, The nature of training response: Peripheral and general adaptations to one-legged exercise Acta physiol. scand. 1973 In press.

...TER, P A J GEROULL B SALMONS and F C. A. ROMANUL, Synthesis by fast muscle of myosin light chains characteristic of slow muscle in response to long-term stimulation. Nature New Biol. 1973. 24, 17–19

...YLOR, A W B Essén and B. SALTIN, Myosin ATPase in skeletal muscle of healthy men. Acta physiol. scand 1974 91 568–570.

Acta physiol. scand. 1975. 95. 166–178

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The Postnatal Development of Some Twitch and Fatigue Properties of the Ankle Flexor and Extensor Muscles of the Cat

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Abstract

HAMMARBERG C and J-O KELLERTH. *The postnatal development of some twitch and fatigue properties of the ankle flexor and extensor muscles of the cat* Acta physiol. scand. 1975 95 166–178

The isometric responses of the medial gastrocnemius (MG), soleus (SOL) and anterior tibial (TA) muscles to single shocks and different modes of repetitive stimulation were studied in kittens of various postnatal ages and in adult cats. The postnatal decrease in time-to-peak and half relaxation time of the twitch contractions was similar for the MG and TA muscles and adult values were attained at around 6–7 weeks of age. The SOL muscle displayed a transient decrease in contraction time during the first postnatal weeks followed later by slowing towards adult values. The susceptibility to fatigue during iterative stimulation was smallest in the SOL at all ages studied, and usually largest in TA. It changed only little for the MG and SOL postnatally while increasing markedly for the TA up until 6–7 weeks of age. Tetanic contractions resulted in similar depressions in contractile tension of all three muscles in the youngest kittens, but SOL displayed a greater ability to recover from this depression than the MG and, in particular, the TA muscles. Tetanus resistance increased postnatally and adult responses were attained at 6–7 weeks of

It has long been recognized (see Ranvier 1874) that the limb muscles of adult mammals may be classified into so-called "fast" and "slow" twitch muscles which differ in their speeds of contraction. As was first described by Banu (1922), however at birth all the limb muscles of the kitten contract slowly the twitch response having a time course similar to that of adult slow twitch muscles. The ensuing process of limb muscle differentiation during the postnatal stages of cat ontogeny has been the subject of several investigations dealing with for example isometric twitch characteristics (Denny Brown 1929 Buller *et al.* 1960 Buller and Lewis 1965) twitch/tetanus ratios (Buller and Lewis 1965 Close and Hoh 1967) force/velocity properties (Close and Hoh 1967) and post tetanic potentiation of peak twitch tension (Buller and Lewis 1965 Nyström 1968 a, f) An additional important aspect of muscle performance which has received less attention is the sensitivity to fatigue during prolonged activity In the adult cat Burke *et al* (1971 1973 1974) found this parameter to be of major significance in the classification of different types of motor units.

The present investigation was undertaken to study the changes in some twitch and fat

perties which occur in the anterior tibial, medial gastrocnemius and soleus muscles of cat during postnatal development. The results obtained also serve as a basis for following siological studies on the postnatal differentiation of various motor unit types in the nkle muscles (Hammarberg and Kellerth 1975 b). The histochemical staining properties hese muscles during postnatal and adult stages have been described previously (Hammar g 1974 a, b).

Methods

experiments were performed on 7 adult cats and 16 kittens varying in ages between 6 and 65 days. animals were anaesthetized with 40 mg/kg of pentobarbitone sodium (Nembutal) given intraperitoneally. Subsequent doses, when necessary, were given through a catheter inserted in the left femoral vein. animals were usually allowed to breathe spontaneously through a tracheal cannula, and expired CO_2 monitored continuously. In one adult animal artificial respiration was employed in order to study the ct of hyper- and hypoventilation upon muscle performance (see Fig. 1 and 2).

he anterior tibial (TA), medial gastrocnemius (MG) and soleus (SOL) muscles of the right hind limb exposed and dissected free except for their proximal attachments, care being taken to preserve their od and nerve supplies. The corresponding motor nerves were cut in the popliteal fossa and mounted on mulating electrodes. In some animals muscles from both legs were used for the experiment. The animals mounted in a steel frame with the long axis of the hind limb almost horizontal. A steel drill transfixed distal end of the femur while the distal ends of the tibia and fibula were rapidly clamped. The cut distal scle tendons were arranged for separate isometric connection to a strain gauge myograph through rt lengths of braided suture (compliance 0.05 mm/kg cm). Care was taken to align the strain gauge h the natural direction of muscle pull. The strain gauge was attached to a sliding metal rod provided h a catch which made it possible to extend the muscles millimetre by millimetre. In order to provide tandard by which to compare the contractions of the different muscles at various postnatal ages, the gth of each muscle was always adjusted to give maximal twitch responses (Buller *et al.* 1960). By elevating surrounding skin flaps it was possible to immerse the exposed tissues in a pool of paraffin oil. Muscle perature was monitored by means of infrared light. With the youngest kittens, however, the small umes of the paraffin oil pools made control difficult and variations in intramuscular temperature ween 34–38°C had to be accepted. Body temperature was kept at 36–38°C.

Muscle contractions were evoked by stimulating the appropriate muscle nerves with supramaximal are wave pulses of 0.3 ms duration. Because of the varying ages of the animals strain gauges of different itional strains had to be used to record the mechanical responses. The unloaded natural resonant frequency the strain gauges exceeded 350 Hz in all cases. The output of the strain gauge was amplified and displayed an oscilloscope and on a penwriter (Devices M 2). The oscilloscope traces were photographed on paper m.

ting procedures

1) Twitch contractions were elicited by single stimulating pulses. The "twitch rise time" was measured from start of the contraction to its peak, while the "twitch half-relaxation time" was measured from the peak the point representing 50 % passive decay in twitch tension. Because the speed of muscle contraction a fairly high temperature coefficient and because muscle temperature was often difficult to control in smallest kittens, the recorded values for contraction time have been adjusted in Fig. 3 to give the corresponding values at 38.0 C by using the correction factor T_{10} 1.55 (Gordon and Phillips 1953, Buller al. 1968). The maximal adjustment made in Fig. 3 amounted to 21 % reduction of the recorded value.

3) *Fatigue properties* of the muscles were examined by the following series of tests:

1. *Fatigue test.* Short trains of stimuli with a stimulus frequency of 40 Hz and a duration of 330 ms were repeated once a second (see Fig. 1 and 2). This test, which will be referred to below as the "fatigue test", was originally introduced by Burke *et al.* (1971) to assess the sensitivity to fatigue of single motor units. The amount of fatigue exhibited by the various muscles after two minutes of stimulation will be expressed as the "fatigue index", i.e. the ratio of the tension output during the 120th tetanus to the tension produced by the first tetanus of the sequence.

2. *Pause test.* After about 2 min of the "fatigue test" stimulation was suddenly interrupted for ten

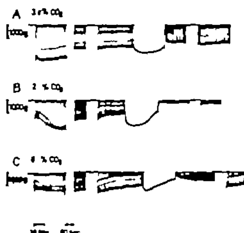


Fig. 1

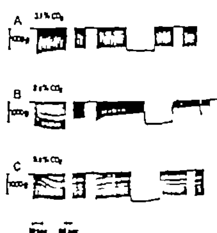


Fig. 2

Fig. 1 and Fig. 2 The contractile responses of the MG (Fig. 1) and SOL (Fig. 2) muscles of a ... during the "fatigue" "tetanus and "pause tests In each record the concentration of expired ... been indicated. *A* Normal breathing. *B*. Hyperventilation. *C* Hypoventilation. It is seen that ... in either direction from normal ventilation will markedly affect the ability of both fast and slow ... to maintain their contractile tensions during sustained activity *B* and *C* were recorded after 5 min ... and hypoventilation, respectively when the new concentration of expired CO_2 had reached ... state. Between *B* and *C* the animal was allowed to breathe spontaneously for 45 min which ... for the complete restitution of normal contractile responses seen in *A*.

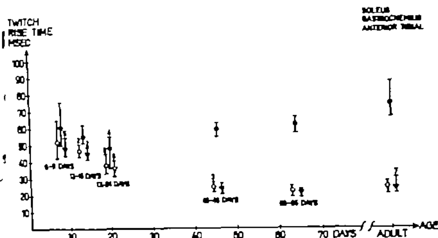
seconds. This is referred to as the "pause test When stimulation was resumed the amount of ... in contractile tension following the short rest was studied (see Fig. 1 and 2).

3 "*Tetanus test* Approximately 30 s after the "pause test when tension output had returned to ... or less steady level the muscle was exposed to 30 s to sustained tetanic stimulation at frequenc... Hz (see Fig. 1 and 2). The iterative stimulation was then resumed and the changes in tension output ... both during and after this so-called "tetanus test were studied.

4. About 30–45 s after the end of the maintained tetanic activation a second pause test was pe... and the ensuing recovery in contractile tension measured.

Results

Twitch rise times (time-to-peak) of the MG SOL and TA muscles at various po... ages are shown in Fig. 3 The results are essentially in agreement with the observati... Buller *et al.* (1960) Buller and Lewis (1965), Mann and Salafsky (1970) and West... *et al.* (1973) In the smallest kittens all three muscles displayed similarly slow contra... although there was a tendency for the SOL muscle to have a somewhat higher mea... in all the age groups studied here. There was a parallel decrease in rise time for the 3 m... during the first 3–4 weeks, after which the SOL rise time increased while the rise ti... the other 2 muscles continued to decrease. It appears, therefore, that the soleus ... contracts faster at around 3 weeks of age than in the adult stage (p 0.002). Th... striking difference between the adult and kitten twitches is seen in the fast MG a... muscles, the adult muscles being markedly faster The adult values for twitch ris... were fairly well acquired by the fast muscles at around 6–7 weeks of age, while those ... SOL muscle were acquired somewhat later (*cf* also Buller *et al* 1960, and Man... Salafsky 1970). In all age groups studied here there was a consistent tendency for t... to contract slightly faster than the MG



3 The postnatal changes in twitch rise time (time-to-peak) for the MG, SOL and TA muscles. Each shows the range of observed values, the number of hich is indicated above each bar. The round and ngular symbols show the mean values

Twitch half-relaxation time (*HRT*) Fig. 4 illustrates the postnatally occurring changes HRT of the three muscles. Here also the fast MG and TA muscles acquired their adult lues at around 6–7 weeks of age, while the SOL possibly took somewhat longer The an values for HRT at different ages are generally consistent with the findings of Buller *al.* (1960) and Mann and Salafsky (1970) except in the case of kittens around 1 week of e, where the present study found the value for SOL in particular to be considerably aller than in the earlier studies (HRT mean values 48, 0 ± 15.5 ms and 35.5 ± 9.9 ms for

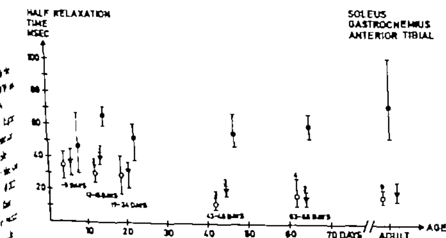


Fig. 4. The postnatal changes in twitch half-relaxation time for the MG, SOL and TA muscles. Each bar indicates the range of observed alues, hile the round and triangular symbols show the mean values. The number of observations is indicated above each bar.

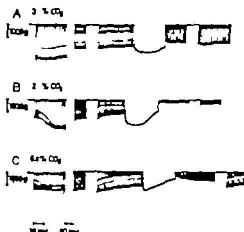


Fig. 1

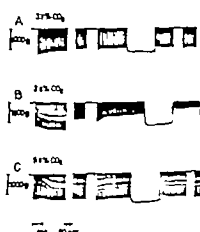


Fig. 2

Fig. 1 and Fig. 2. The contractile responses of the MG (Fig. 1) and SOL (Fig. 2) muscles of an adult during the "fatigue tetanus and "pause tests In each record the concentration of expired CO_2 been indicated. *A* Normal breathing. *B.* Hyperventilation. *C* Hypoventilation. It is seen that deviations in either direction from normal ventilation will markedly affect the ability of both fast and slow muscles to maintain their contractile tensions during sustained activity *B* and *C* were recorded after 5 min of hyper- and hypoventilation, respectively when the new concentration of expired CO_2 had reached a steady state. Between *B* and *C* the animal was allowed to breathe spontaneously for 45 min which was sufficient for the complete restitution of normal contractile responses seen in *A*.

seconds. This is referred to as the pause test When stimulation was resumed the amount of recovery of contractile tension following the short rest was studied (see Fig. 1 and 2).

3. "*Tetanus test* Approximately 30 s after the pause test when tension output had returned to a more or less steady level, the muscle was exposed for 30 s to sustained tetanic stimulation at a frequency of Hz (see Fig. 1 and). The iterative stimulation was then resumed and the changes in tension output occurring both during and after this so-called "tetanus test were studied.

4 About 30–45 s after the end of the maintained tetanic activation a second pause test was performed and the ensuing recovery in contractile tension measured

Results

Twitch rise times (time-to-peak) of the MG SOL and TA muscles at various postnatal ages are shown in Fig. 3 The results are essentially in agreement with the observations of Buller *et al* (1960), Buller and Lewis (1965) Mann and Salafsky (1970) and Westerman *et al* (1973). In the smallest kittens all three muscles displayed similarly slow contractions although there was a tendency for the SOL muscle to have a somewhat higher mean value in all the age groups studied here. There was a parallel decrease in rise time for the 3 muscles during the first 3–4 weeks, after which the SOL rise time increased, while the rise times of the other 2 muscles continued to decrease. It appears, therefore, that the soleus muscle contracts faster at around 3 weeks of age than in the adult stage ($p < 0.002$). The most striking difference between the adult and kitten twitches is seen in the fast MG and TA muscles, the adult muscles being markedly faster The adult values for twitch rise time were fairly well acquired by the fast muscles at around 6–7 weeks of age, while those of the SOL muscle were acquired somewhat later (*cf* also Buller *et al* 1960, and Mann and Salafsky 1970). In all age groups studied here there was a consistent tendency for the TA to contract slightly faster than the MG

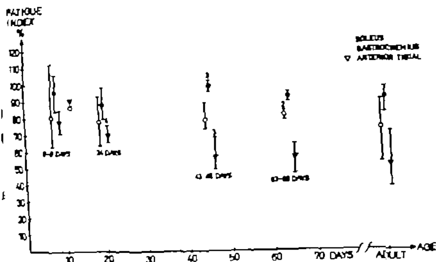


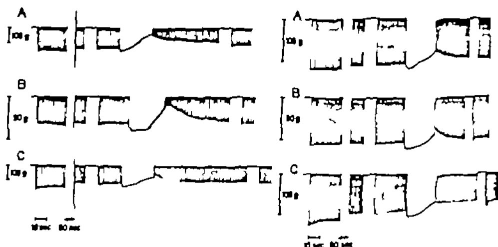
Fig. 6 The postnatal changes in "fatigue index" for the MG, SOL and TA muscles. Each bar illustrates the range of observed values, while the round and triangular symbols show the mean values. The number of observations is indicated above each bar

...ion of stimulation, although earlier in the sequence an initial increase reminiscent of post-tetanic potentiation had gradually disappeared.

In the 18-day-old kitten a qualitatively similar "fatigue test" picture was obtained: both MG and, in particular, the TA showed a progressive reduction in tension output during the 2 min of stimulation, while the SOL remained virtually unaffected. The same general tendency was present in the 48-day-old kitten, as well as in the adult animal.

Fig. 6 illustrates the *fatigue indices* (see Methods) for each of the tested muscles at various postnatal ages. The change in fatigue index occurring during postnatal development was quite conspicuous in the case of the TA muscle which became less resistant to fatigue with increasing age ($p < 0.01$). In the MG and SOL muscles, however, the changes were insignificant, and the relative difference in mean values between the two muscles remained rather constant across the age groups investigated. In the adult animal a clear difference in fatigue index was found between the TA and SOL muscles ($p < 0.001$), while MG appeared to occupy a more intermediate position.

Tetanus test. Although the "fatigue indices" (Fig. 6) suggest that both the SOL and MG muscles maintain a more or less unchanged resistance to fatigue throughout the postnatal period, a different picture is obtained when the "tetanus test" is used. As seen in Fig. 5, all 3 muscles of the 7-day-old kitten showed considerable reduction in tension during the course of the 30 s long tetanic stimulation. Generally, in this age group, the three muscles tested showed similar amounts of reduction in tension. At 18 days of age the ability to withstand the tetanus had improved, but no obvious differences were yet observed among the individual muscles. At around six to seven weeks of age, however, a pattern had emerged which strongly resembled that of the adult animal. In the SOL muscle the tetanic tension was



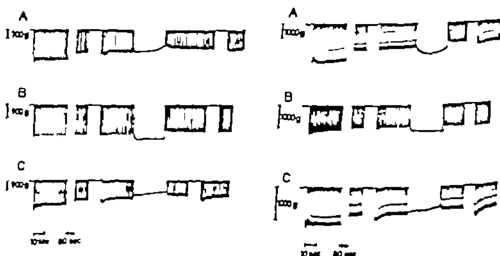


Fig. 5 Typical examples of the contractile responses of the MG (*A*) SOL (*B*) and TA (*C*) muscles d the fatigue tetanus and pause tests at different postnatal ages

SOL and MG respectively compared to approximately 70–75 ms and 40–45 ms, res tively according to Fig. 4 of Buller *et al* 1960). With respect to the general pattern of mat tion the contraction times (Fig. 3) and half-relaxation times (Fig. 4) for the different mu were found to display similar time courses (see also Buller *et al* 1960).

"Fatigue test" Fig. 5 shows some typical examples of the behaviour of the MG, S and TA muscles (A, B and C respectively) at different postnatal ages during prolo activity

The MG and TA muscles (A and C, respectively) of the 7-day-old kitten of Fig. 5 sho a moderate reduction in contractile tension after 2 min of intermittent stimulation, reduction being somewhat more pronounced for the latter muscle. The SOL muscle on the other hand, produced approximately the same amount of contractile tension :

...s it has been shown that such differences are reflected in the size of their vascular sup-...s (Romanul 1965). Alternatively these effects might also be caused by extracellular ...umulation of potassium or metabolites in the muscles (Lüttgau 1965).

...he *post-tetanic period*" which followed the 30 s tetanus, also deserves comment. In the ...eek-old kittens (see Fig. 5) the tetanus always induced a considerable initial reduction ...the amplitude of the following repetitive contractions. This reduction usually was of ...ilar magnitude for the 3 muscles tested. It did not persist, however but a gradual recovery ...contractile tension started almost immediately and proceeded to a certain plateau. Al...ugh the total amount of recovery achieved by a particular muscle varied somewhat be...en animals, it was consistently found to be largest in the SOL muscle and usually smallest ...he TA muscle. In the 2- to 3-week-old kittens the initial depression in contractile strength ...sed by the tetanus had decreased in all three muscles, but the strong tendency for recovery ...the SOL muscle was still evident. The TA muscle generally had a poorly developed re...ery ability at this age, while the MG showed a beha...iour intermediate to the other two ...scles. In the 6- to 7-week-old kittens and adult animals the initial depression in contractile ...ength following the tetanus was either absent or very small, indicating that the muscles ...ched their post-tetanic plateaus quickly Typically in these age groups post-tetanic SOL ...ntractions had approximately the same amplitude as contractions before the tetanus, and ...s TA contractions were generally somewhat more depressed post-tetanically than the ...G ones (*cf* Fig. 5).

It appears, therefore, that with regard to the changes in contraction strength which occur ...ring the "post-tetanic period" a difference between the muscles is already present at 7 ...s of age. The SOL muscle exhibited a larger post-tetanic recovery than the other muscles, ...ile the TA contractions were usually more depressed than those of the MG Qualitatively ...e same tendencies remained throughout the different age groups studied.

The *pause test* was applied to each muscle both before and after the "tetanus test ...e intention was to study the amount of recovery in contractile tension immediately after ...s rest. In general, the second of these pause tests" was found to be more sensitive than ...e first in displaying possible differences in recovery properties between the various muscles ...f Fig. 5). This would be expected since particularly in the youngest kittens the muscles ...peared to be less exhausted after 2 min of iterative stimulation than after the later "tetanus ...st (see Fig. 5). Fig. 8 illustrates for each muscle at various postnatal ages the relative ...mount of recovery in contractile tension following the second pause test which was ...lated about 30–45 s after the end of the tetanus. Although a considerable spread of values ...as found for muscles of the same postnatal age the mean ...alues (±S.E.) for recovery were ...lmost the same at one week postnatally (MG 15.3±6.2%, TA 25.4±13.0%, SOL 5.4± ...0%) as at the adult stage (MG 14.5±8.4...%, TA 28.7±9.0%, SOL 6.5±6.0%). An im...ortant finding which is not evident from Fig. 8 is that in all the animals studied there was ...nly one exception (the 7-day-old kitten of Fig. 5) to the general rule that the TA muscle ...lways exhibited the largest amount of recovery in contractile tension after the pause, while ...he SOL muscle always showed ... recovery which was less than that of MG. This increase ...n TA contractile tension after the pause was usually short-lasting, however and disappeared ...radually during the following 10–20 ... of iterative stimulation.

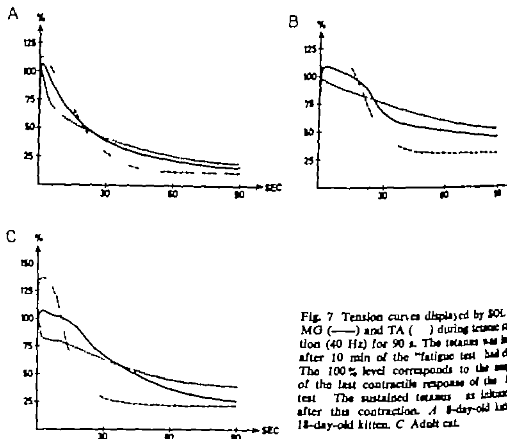


Fig. 7 Tension curves displayed by SOL MG (——) and TA ( ) during tetanic st tion (40 Hz) for 90 s. The tetanus was in after 10 min of the "fatigue test" had el The 100% level corresponds to the amp of the last contractile response of the test The sustained tetanus as ... after this contraction. A 8-day-old kit 18-day-old kitten. C Adult cat.

well maintained throughout the period of stimulation and, furthermore, its magnitude al exceeded the tension produced by the intermittent stimulation by 10%–40%. In th muscle the tetanic tension declined continuously throughout the stimulation period a initial peak amplitude never exceeded the contractile tension produced by the prec intermittent activation The MG muscle usually showed a gradual increase in tensio 10–15 s, followed by a smooth decline. In the adult animals the peak of this rather co tension curve always represented a tension exceeding that produced by the iterative st lation immediately prior to the tetanus.

In a few animals the continuous tetanic stimulation was not started until 10 min a "fatigue test" had elapsed It was then maintained for 90 s. As seen in Fig. 7 the SO all ages was more depressed by this kind of stimulation than were the other two mus although the difference between the muscles was rather small in the youngest kittens. T results may appear to be in disagreement with those of the "tetanus test" (see above) wh particularly in the older animals, the SOL was always found to be more fatigue resi than the MG and TA muscles (see Fig. 5) However since the pattern of fatigue illustr in Fig. 7 was never observed when studying single motor units of different types (Ham berg and Kellerth 1975 a, b) these results may be interpreted as being due to ischa effects caused by occlusion of the blood supply of the muscles resulting from the l lasting tetanic contraction (cf Emmelin and MacIntosh 1956). The fatigue curves of F would then rather be taken to display differences in aerobic metabo sm among the mus

orted by Buller *et al.* (1960), possibly due to small temperature differences (Gordon and llips 1953, Buller *et al.* 1968).

A gradual decrease in contraction time of the SOL was observed during the first 3 postal weeks, followed by an increase approaching adult values which were achieved later n in the case of the fast muscles (see also Buller *et al.* 1960 Mann and Salafsky 1970). s interesting to note, in this connection, that histochemical studies have demonstrated riation in staining patterns among fibres of the MG, TA and SOL muscles up until 3–4 ks postnatally after which age SOL alone gradually loses its differentiated character l attains its adult homogeneous appearance by 7 10 weeks postnatally (Karpati and Engel 7 Nystrom 1968 a, Hammarberg 1974 b).

The pattern of postnatal changes in HRT is generally consistent with that described by vious authors (Buller *et al.* 1960, Mann and Salafsky 1970) except in the case of the ngest kittens, where the soleus HRT in particular was found to be shorter than exted. The gradual decrease in soleus HRT which has been reported by others to occur ring the first 3 postnatal weeks (*e.g.* Buller *et al.* 1960) was therefore not substantiated in present study nor in a subsequent investigation of single motor units (Hammarberg l Kellerth 1975 b). We cannot at this point, however offer any satisfactory explanation the discrepancy between these results. The HRT of the MG and TA was found to de ase directly with the contraction time, the adult values being attained at around 6–7 eks postnatally

The "fatigue indices observed in adult animals suggest that differences exist among the ee mature muscles in terms of their sensitivity to fatigue, SOL being least susceptible to igue and TA most susceptible. These findings probably reflect differences in the mature scles with regard to their relative composition by various functional types of motor its (Burke *et al.* 1973 1974, Proske and Waite 1974, Hammarberg and Kellerth 1975 a). e relatively homogeneous composition of SOL with respect to muscle fibre physiology d histochemistry has been verified repeatedly (see *e.g.* Henneman and Olsson 1965 Nyöm 1968 a, Burke *et al.* 1974 Hammarberg 1974 a). It would also appear reasonable that e MG as an antigravity muscle would contain a greater proportion of fatigue resistant its than the TA, which is engaged more in phasic movements with little load. In the ungest kittens the three muscles exhibited similar "fatigue indices as would be expected ce muscle fibres are less differentiated at this age (Banu 1922, Denny Brown 1929 uller *et al.* 1960, Nystrom 1968 a, Hammarberg 1974 b). The TA muscle showed a marked crease in sensitivity to fatigue with increasing age, adult values being attained at around 7 eeks postnatally The MG and SOL muscles, on the other hand, maintained more or ss unchanged "fatigue indices throughout the developmental period.

It should be pointed out, however that with prolonged stimulation changes in amplitude contractile responses are probably determined by two factors with opposite effects, amely potentiation and fatigue (v. Euler and Swank 1940). In the smallest kittens potentiation following low-frequency tetanization has been found to be more prominent in the OL than in the MG, while the reverse situation was found in adult animals (Standaert 964, Nystrom 1968 a, f see also Burke *et al.* 1973 1974). If the possible contribution of otentiation is taken into account, one cannot exclude the possibility that the differences

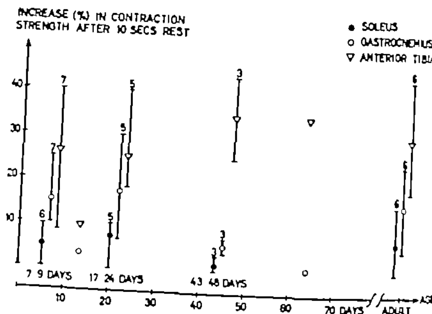


Fig. 8 The relative increase in contract le strength occurri g in the MG SOL and TA musc of the second pause-test plotted against postnatal age. The O -ordinate corresponds to th of the last contraction prior to the pause. Each bar illustrates the range of observed values, whi and triangula symbols show the mean values. The umber of observations is indicated abov

These findings suggest in summary that with regard to the pattern of recover tractile tension following the "tetanus test" differences already exist between p fast and slow hind limb muscles after the first postnatal week. The SOL muscle a marked ability to overcome post tetanic depression in contractile tension, and was usually more or less complete *before* the "pause test" The fast muscles, and cular the TA, remained significantly more depressed post tetanically and even wh by a 10 s rest only temporarily and to a limited degree approached the pre-tetanic contractile tension. The possible mechanisms underlying this early difference betw muscles will be discussed below

Discussion

The present study has been confined to an investigation of the changes in some tw fatigue properties which occur in the MG SOL and TA muscles of the cat during th natal period. The developmental changes in contraction time have previously been re by several other investigators (Banu 1922, Denny Brown 1929 Buller *et al* 1960, and Lewis 1965 Mann and Salafsky 1970, Westerman *et al* 1973), and were include only for comparison with the pattern of changes in fatigue properties observed in the animals.

The postnatal changes in contraction time of the potentially fast MG and TA m were found to follow a time course similar to that reported by previous authors (*e.g* B *et al.* 1960, Buller and Lewis 1965) and adult values were recorded at 6–7 weeks of age contractions were generally somewhat faster particularly in the smallest kittens, than

...ration of the flexor motor system (Kellerth *et al.* 1971 Mellström 1971 Hammarberg ... b) also appear incompatible with the view that this system would be more apt to fail ...synaptic spike propagation or in neuromuscular transmission than would the extensor ...m. In our opinion, therefore, it seems more likely that the observed differences in ...e properties of the immature muscles will be found postsynaptically where differences ...ost-tetanic potentiation have been shown to exist (Nyström 1968 f).

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References

...G. *Recherches physiologiques sur le développement neuromusculaire.* Paris 1932.

...A. J. C. Eccles and R. M. Eccles, Differentiation of fast and slow muscles in the cat hind ...b. *J. Physiol.* (Lond.) 1960. *150.* 399–416.

...A. J. and D. M. Lewis, The rate of tension development in isometric tetanic contractions of ...mammalian fast and slow skeletal muscle. *J. Physiol.* (Lond.) 1965. *176.* 337–354.

...A. J. K. W. Ranatunga and J. M. Smith, The influence of temperature on the contractile ...characteristics of mammalian fast and slow twitch skeletal muscles. *J. Physiol.* (Lond.) 1968. *196.* 83P

...R. E. D. N. Levine, F. E. Zajac, III, P. Tsairis and W. K. Engel, Mammalian motor units. ...physiological-histochemical correlation in three types in cat gastrocnemius. *Science* 1971. *174.* 709–712.

...R. E. D. N. Levine, P. Tsairis and F. E. Zajac, III, Physiological types and histochemical profiles ...motor units of the cat gastrocnemius. *J. Physiol.* (Lond.) 1973. *234.* 723–748.

...R. E. D. N. Levine, M. Saloman and P. Tsairis, Motor units in cat soleus muscle: Physiological, ...biochemical and morphological characteristics. *J. Physiol.* (Lond.) 1974. *238.* 503–514.

...R. I. and J. F. Y. Hou, Force: velocity properties of kitten muscles. *J. Physiol.* (Lond.) 1967. *192.* 15–822.

Brown, D. E. The histological features of striped muscle in relation to its functional activity ...roc. roy. Soc. *B* 1929. *104.* 371–411.

...F. T. The blockage of motor impulses in an asynchronized volley at the neuromuscular junction. ...comp. Physiol. 1953. *46.* 348–350.

...N. and F. C. MacIntosh, The release of acetylcholine from perfused sympathetic ganglia and ...skeletal muscles. *J. Physiol.* (Lond.) 1956. *131.* 477–496.

...U. S. and R. L. Sw..., Tension changes during tetanus in ... and ... muscle. ...Acta physiol. scand. 1940. *1.* 203–219.

...T. P. Studies on the neuromuscular junction. IV. The nature of junctional inhibition. *Clin. J.* ...Physiol. 1937. *11.* 437–450.

...G. and C. G. Phillips, Slow and rapid components in ... flexor muscle. *Quart. J. exp. Physiol.* ...*33.* 5–43.

...C. Histochemical staining patterns of muscle fibres in the gastrocnemius, soleus and ...anterior tibial muscles of the adult cat, as viewed in serial sections stained for lipids and succinic de...hydrogenase. *Acta Neurol. Scandinav.* 1974. *50.* 272–284.

...C. *The histochemical appearance of developing muscle fibres in the gastrocnemius, soleus* ...and anterior tibial muscles of the kitten, as viewed in serial sections stained for lipids and succinic ...dehydrogenase. *Acta Neurol. Scandinav.* 1974 b. *50.* 285–301.

...C. and J.-O. Kellerth, Studies of some twitch and fatigue properties of different motor ...unit types in the ankle muscles of the adult cat. *Acta physiol. scand.* 1975 a. *95.* 231–242.

...C. and J.-O. Kellerth, The postnatal development of some twitch and fatigue properties ...of single motor units in the ankle muscles of the kitten. *Acta physiol. scand.* 1975 b. *95.* 243–257.

...E. and C. B. Olson, Relations between structure and function in the design of skeletal muscles. ...J. Neurophysiol. 1965. *28.* 581–598.

...G. and W. K. Engel, Neuronal trophic function. *Arch. Neurol.* 1967. *17.* 542–545.

...J.-O. A. M. ...ström and S. Skoglund, Postnatal excitability changes of kitten motoneurones. ...*Acta physiol. scand.* 1971. *83.* 31–41.

in "true" fatigue between the muscles may be smaller in the youngest kittens and larger in the adults than the "fatigue indices" of the present study suggest. Such a possibility is supported by the results of the "tetanus test" where the intention was to produce fatigue in the immature muscles in order to compensate for any simultaneous potentiation effects. In this situation, all three muscles of the youngest kittens were found to respond in a similar way and the postnatal changes in their tetanus responses also followed a similar time course, the adult pattern being attained at around 6–7 weeks postnatally (see Fig. The potentiation which would be expected to occur as a consequence of the prolonged stimulation during this "tetanus test" (Nyström 1968 a, f) may also help explain the differences in contractile behaviour of the immature muscles during the "post-tetanic period". In the youngest kittens the SOL muscle showed a marked tendency to recover from the post-tetanically depressed state of tension output, possibly due in part to strong PTP at this particular age (Nyström 1968 a, f). With increasing age the SOL gradually improved its resistance to fatigue despite the simultaneous disappearance of potentiation (Nyström 1968 a, f). The fast muscles, on the other hand, remained quite depressed post-tetanically in the smallest kittens, due possibly to the lack of potentiation at this age (Nyström 1968 a, f). However, even when supported by potentiation these muscles in the adult animal cannot match the fatigue resistance of the SOL (Standaert 1964, Nyström 1968 a, f, see also Burke *et al.* 1973, 1974).

The term "fatigue" has been used in a rather unspecific sense in the present study to denote merely the decrease in contractile tension which occurs after prolonged stimulation. Obviously, deficient contractions may result from failure of any one of several mechanisms, and the type of failure may even be different in immature kittens and adult cats. The possibility of failure of initiation or propagation of impulses in the nerve stem fibres appears unlikely, however, since stimulus strength was always kept supramaximal. Furthermore, when the same stimulus frequencies were used in studies on single motor units, a 1:1 input-output relationship was always found to exist between stimuli and EMG responses in both kittens and adult cats, even during fatigue (Burke *et al.* 1973, 1974, Hammarberg & Kellerth 1975 b). However, the present results cannot exclude the possibility of a partial presynaptic failure of propagation involving more distal parts of an axon, such as any of its fine nerve terminals (Ramsay and Street 1942, Dun 1955, Krnjević and Miledi 1958), a failure of neuromuscular transmission (Feng 1937, Rosenblueth and Morison 1937, Krnjević and Miledi 1958, Shamarina 1961) or a postsynaptic failure of electrical and contractile mechanisms in the muscles (Merton 1954, Krnjević and Miledi 1958, Luff 1965). There exists some evidence, however, that the observed *differences* in fatigue properties between the potentially fast and slow muscles of the youngest kittens (see Figs. and 8) may not be accounted for by corresponding differences in presynaptic mechanisms or in neuromuscular transmission. During the first 3–4 postnatal weeks the SOL and M have previously been found to appear quite similar with respect to a number of parameters such as fibre diameter distribution and conduction velocity of the efferent nerve fibres, relationship between muscle fibre diameters and size of nerve terminals, morphology of the motor end plates, ACh-esterase activity, and increase in transmitter mobilization following conditioning tetanization (Nyström 1968 b–e). Furthermore, previous ... tions of an early

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The Influence of Rheological and Collapse Factors on Pre- and Post-Capillary Flow Resistances in the Skeletal Muscle Vascular Bed of the Cat

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Abstract

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…importance of passive-elastic changes of vascular dimensions and shifts in regional effective viscosity …he pre- postcapillary resistance ratio (R_A/R), and hence mean capillary pressure (P_C), was analysed …t calf muscle. Reductions of mean venous distending pressure below 6–8 mm Hg induced marked R… …ases due to escalating venous collapse. This mechanism tends to defeat the P reductions and rate …transcapillary fluid absorption during intense precapillary vasoconstriction. Comparisons of R_A/R_V …erythrocyte suspensions (Hct 40–50) and cellfree perfusates at identical vascular dimensions showed … R_A/R …as considerably higher and P correspondingly lower for the erythrocyte suspension except …ery low flows. This R_A/R_V difference increased with increasing flow and at very high flows P was …ut 10 mm Hg lower during perfusion …th the erythrocyte suspension. These findings apparently diverge …n the know… influence of tube radius and linear flow velocity on effective …e …ave viscosity of blood. …e distal precapillary and proximal capillary sections, both having smaller diameters than the erythro…c…, are located upstream to the point of filtration–absorption equilibrium, they contribute to this respect R… It is therefore suggested that the increasing R… /R_V with erythrocyte perfusion, particularly at higher …ws, … not due to genuine viscosity factors but to friction losses when cells in "bolus flow" are squeezed …ough the narrowest precapillary sections.

…e resistance to blood flow in the …ascular bed is, according to the Poiseuille relation, …termined by the …essel dimensions and the effective viscosity of the blood. Several …udies (…g. Whittaker and Winton 1933 Djojosugito *et al.* 1970), where blood viscosity …re was determined at varying flow rates, indicate that effective blood viscosity is …rprisingly low considering the high content of suspended red corpuscles. Thus, in the …ximally dilated muscle …ascular bed Djojosugito *et al.* (1970) observed an effective …scosity of 2.3–2.5 cP at high blood flows; that is, only about twice the value for plasma …scosity Furthermore, *in vi*… blood …iscosity increases surprisingly little even when flow … markedly reduced by …asoconstriction, then rarely exceeding 3 cP *i.e.* only 30% higher

…On paid leave of absence from the MRC Toxicology Unit, England.

KANJEVIĆ, K. and R. MILEDI, Failure of neuromuscular propagation in rats. *J Physiol.* (Lond.) *140* 440–461

LÜTTGAU H C., The effect of metabolic inhibitors on the fatigue of the action potential in single fibres. *J Physiol.* (Lond.) 1965. *178*. 45–67

MANN, W S. and B. SALAFSKY Enzymic and physiological studies on normal and denervated developing and slow cat muscles. *J Physiol.* (Lond.) 1970. *208* 33–47

MELLSTRÖM, A., Postnatal excitability changes of the ankle monosynaptic reflexes in the cat. *Acta physiol. scand.* 1971 *82*. 477–489

MERTON, P A., Voluntary strength and fatigue. *J Physiol.* (Lond.) 1954. *123*. 553–564.

NYSTRÖM, B., Mechanical and electrical responses to single shocks in developing cat leg muscles [illegible] tetanization. *Acta physiol scand.* 1968 a. 74. 207–225.

NYSTRÖM, B., Fibre diameter increase in nerves to "slow red" and "fast white" cat muscles during postnatal development. *Acta Neurol. Scandinav.* 1968 b. *44* 265–294

NYSTRÖM, B. Postnatal development of motor nerve terminals in "slow red" and "fast-white" cat muscles. *Acta Neurol Scandinav.* 1968 c. *44* 363–383

NYSTRÖM, B. Histochemical studies of end plate bound esterases in "slow-red" and "fast-white" muscles during postnatal development. *Acta Neurol. Scandinav.* 1968 d. *44* 295–318.

NYSTRÖM, B., Post tetanic decurarization in developing cat leg muscles. *Acta physiol. scand.* 1968 [illegible] 226–237

NYSTRÖM, B., Effect of direct tetanization on twitch tension in developing cat leg muscles. *Acta physiol. scand.* 1968 f *74* 319–339

NYSTRÖM, B., Histochemistry of developing cat muscles. *Acta Neurol. Scandinav.* 1968 g. *44* 405–4[illegible]

PROSKE, U and P M E. WAITE, Properties of types of motor units in the medial gastrocnemius [illegible] of the cat. *Brain Res* 1974 *67* 89–101

RAMSEY R W and S. F STREET Absence of fatigue of the contractile mechanism in single muscle [illegible] *Fed Proc* 1942. *1* 70.

RANVIER, L., De quelques faits relatifs à l'histologie et à la physiologie des muscles striés. *Arch. Physiol Norm. et Pathol* 1874. *6*. 1 15

ROMANUL, F C. A., Capillary supply and metabolism of muscle fibers. *Arch. Neurol.* 1965. *12* 49[illegible]

ROSENBLUETH A and R. S. MORISON Curarization, fatigue and Wedensky inhibition. *Amer J. Physiol.* 1937 *119* 236–256.

SHAMARINA, N M Postsynaptic response of a single muscle fibre to indirect stimulation. *Sechenov physiol. J. USSR* (Engl. Transl). 1961 *47* 1145–1155

STANDAERT F G Th mechanisms of post tetanic potentiation in cat soleus and gastrocnemius [illegible] *J gen. Physiol.* 1964. *47* 987 1001

WESTERMAN, R A D M LEWIS, J BAGUST G EDJTEHADI and D PALLOT Communication between nerves and muscles. Postnatal development in kitten hindlimb fast and slow twitch muscle. In *Memory and Transfer of Information* Ed. H P Zippel, Plenum Publ. Corp., London. 1973. 255–291

[illegible] perfusion

[illegible] preparations were left with an intact arterial blood supply via the femoral artery. In these experiments the femoral artery was cannulated and connected to a pressure reservoir, containing an artificial [illegible] perfusate (4–6 % dextran-Tyrode solution or 6–10 % Ficoll-Tyrode solution). The osmolarity [of the p]erfusates was adjusted to the same level as the cat's own blood, i.e. usually around 300–310 mOsm/l. [Usi]ng clamps alternately on the tubing from the reservoir and on the femoral artery, the perfusion [of the c]alf could be rapidly shifted from blood to Newtonian fluid. By adjustments of a screw clamp on the [femora]l artery or of the reservoir pressure, the inflow pressure could be exactly controlled.

[In o]ther preparations the femoral artery was divided and its peripheral end connected to two pressure [reservoi]rs, one containing 6 or 10 % Ficoll-Tyrode solution, the other erythrocytes, suspended in the same [Ficoll-]Tyrode solution. The reason for using Ficoll instead of dextran solution as a vehicle for the erythro[cytes w]as that their suspension stability was very good in Ficoll, the sedimentation rate in them less than [1 mm/]h. This is in contrast to the situation when erythrocytes are suspended in dextran, in which case the [sedimen]tation rates are extremely high. Erythrocytes were obtained by exsanguination of a heparinized [donor] animal. During the bleeding large amounts of Ficoll were given to allow for effective exsanguination. [The b]lood was collected in a glass cylinder, containing glass beads, and shaken vigorously to get rid of the [platele]ts. The blood was centrifuged and the red cells resuspended in Ficoll, this was repeated 3 times and [after] the last centrifugation the haematocrit was adjusted to 35–40 %. This suspension was passed through [a filter] to remove any clots before it was placed in the pressure reservoir. The reservoirs containing the per[fusates] were placed in a water bath at 38°C. In these preparations, where the perfusion throughout occurred [from] pressure reservoirs, the femoral artery and vein were divided proximal to the site of cannulation, [the n]erve was severed and the calf thus completely separated from the animal.

[Record]ings of circulatory variables

[Arter]ial inflow (P [illegible]) and venous outflow (P [illegible]) pressures were recorded with Statham pressure transducers, [carefu]lly calibrated and repeatedly checked during the experiment. Recordings were made on a Grass [poly]graph. Volume (weight) changes were recorded by means of modified force displacement transducers, [the s]ensitivity of the recording system was set so that 0.1 ml (0.1 g) made a deflection of around 10 mm [on t]he polygraph. — *In vitro* viscosity of blood and other perfusates was measured with an Ostwald visco[simete]r, the osmolality of the perfusates was measured in an osmometer (Advanced Instruments Inc.).

[Expe]rimental procedures

[The] preparation was initially perfused with the artificial, cell-free, oxygenated perfusate. Great care was [take]n to maintain arterial inflow pressure low throughout to avoid oedema formation in the maximally [dilat]ed vascular bed. Early in the experiment the inflow and outflow tubings were temporarily clamped [so t]hat no circulatory exchange could occur, thus checking for leakage from the plethysmograph, bleeding [and/or] evaporation from the preparation and drifts in the recording system. On resuming the perfusion, [P [illegible]] and P [illegible] were adjusted so that an isovolumetric (isogravimetric) situation was obtained. The capillary [pres]sure in this situation, P_{ci}, was then determined according to a modification of the technique by Pappen[hei]mer and Soto-Rivera (1948), earlier described in detail (Elmaniou et al. 1974). First P_A, and then P_V, [wer]e separately elevated to levels causing identical P [illegible] increases, as judged from the induced filtration rate. [Fr]om this measure the pre/postcapillary resistance ratio, and hence P_{ci} can be determined. The capillary [filt]ration coefficient (CFC) was subsequently determined by elevating P [illegible] and P_V, and hence also Pc [by] the same amount, recording the filtration rate as volume or weight gain per time unit.

By adjusting P [illegible] and P [illegible] perfusate flow was then varied over a fairly large range, although the highest [pre]ssures and flows were avoided except for short periods, to minimize oedema formation. The P [illegible] value [fo]r each pair of P [illegible] and P [illegible] levels could be determined as the rate of volume (weight) change (from the [iso]volumetric state) and from the known CFC. To ensure that these deductions remained valid, the P_{ci} [an]d CFC were repeatedly measured during the perfusion period. — With knowledge of the volume flow [P [illegible]] and P [illegible] the pre- and postcapillary resistances (R_A and R_V, respectively) during perfusion with [N]ewtonian fluid could be determined. The dependence of R [illegible] and R [illegible] on the mean arterial and venous [di]stending pressures (P [illegible] P [illegible] 2 and P [illegible] P_V/2 respectively) could thus be calculated.

After making the above measurements with the cell-free perfusate, perfusion was switched to blood or [er]ythrocyte suspension. R_A, R_V, P [illegible] and CFC were again determined in the isogravimetric state and during [v]arying flow rates with calculations of the respective R [illegible] and R [illegible] values from CFC and rate of weight

than the value obtained at maximal flows (Bæckström *et al* 1971). These observ suggest that changes in viscosity are not a normally important determinant of blood and overall resistance, provided the rheological properties of the blood are not dal as in trauma, infection, shock, *etc*

This does not mean, however that changes in blood viscosity are always of lit portance in the control of circulatory homeostasis. For example, the anomalous properties of blood, meaning that its viscosity increases with increasing tube dimensio decreasing flow rates suggest that effective blood viscosity would be higher in the wid post-capillary vessels than in the narrow precapillary vessels, and particularly so when reduced *e g* after neurogenic precapillary constriction If this were true the higher viscosity would by its further relative increase, counteract the increase in pre/postc resistance ratio caused by the luminal changes and perhaps even increase capillary p as a net result If so these differences in effective blood viscosity within the pre- an capillary vascular compartments might even promote filtration and hence reduce the volume.

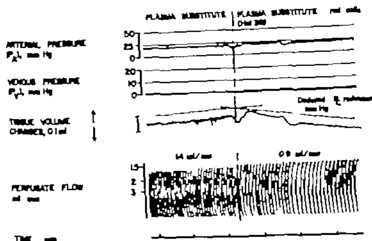
For these reasons it was considered of interest to estimate separately effectiv viscosity in the pre and postcapillary vascular sections. This was done by compari resistances in the two sections when they were perfused with either blood or a New non-corpuscular perfusate during conditions when vascular dimensions were rig controlled This approach also allowed for a detailed analysis of the passive shifts and postcapillary resistances resulting from alterations of the transmural pressure as the viscosity studies are concerned the results went, to our initial surprise, in the o direction to what was theoretically predicted as will be described below — Parts work has been reported earlier (Öberg 1974)

Methods

Experiments were performed o 19 cats anaesthetized with chloralose (50–60 mg/kg b t). The calf muscle preparation, earlier described in detail (g Djojosugito *et al.* 1970, Bæckström et was used. Briefly the calf was isolated from the ormal w th exception of the femur the femoral vein. A hole was drilled in the femur and the ca ty pl gged w th cotton wool to a oid collater circulation from the calf ia the bone marr w Two sid branches to the femoral artery dis calf muscles were cannulated, ne t provide for measurements of arterial nflow pressure, the allow for i.a. infusions of vasodilat drugs. A side branch of the femoral en was used for ments of venous outflow pressure. The femoral ein was cannulated proximally to the pressure and the venous outflow di ected through a d op-recorder and recorded by an ordinate riter on to polygraph recorder The blood (perfusate) flow was checked repeatedly by collecting the outf graduated test tube. Venous outflow pressure co ld be varied by adjusting the height of the free e outflow catheter above the preparation.

Sustained maximal vasodilatation o the preparation wa nduced by infusing intraarterial papaverine (1 mg/min) or nitroprusside sodium (50 / g/mi), dissolved the same plasma sub used for the perfusions (see below)

The preparation was then placed either in plethysmograph for recording changes in tissu (g Kjellmer 1964) or on a sensit balance for record g changes tissue weight When the ex muscle preparation was recorded the skin was cautiously removed w th l gation of all ts blood and the muscles were protected from ev poration by thin layer of silicone grease covered by pol envelope, and from cooling by a heating lamp.



2. The effect of changing perfusate from cell-free plasma substitute (6% Ficoll) to the same plasma substitute to which red blood cells were added to haematocrit of 39%. Note the reduction of flow despite unchanged pressure head when the cell suspension enters the vascular bed, reflecting an increased effective viscosity and the change of volume record from filtration to an absorption slope, indicating reduction capillary pressure. The capillary pressure fall was estimated to amount to 3 mm Hg.

...icating a gradual passive elastic recoil of the precapillary resistance vessels. Similarly postcapillary resistance increases almost linearly until transmural pressure reaches 6–8 mm Hg but upon further pressure reductions disproportionate increases of venous resistance occur.

In all likelihood this latter phenomenon is due to increasing venous collapse, *i.e.* a change of venous cross-sectional geometry to an increasingly elliptical shape. *In vitro* experiments on veins illustrate that such a change of geometry starts when transmural pressure falls below 6–8 mm Hg (*e.g.* Öberg 1967). This marked increase of postcapillary resistance at low transmural pressures means that the pre- and postcapillary resistance ratio (R_A/R_V) decreases sharply in this pressure range. This is demonstrated in the right part of Fig. 1.

The present data thus indicate that the postcapillary resistance is drastically increased by such venous collapse when the mean distending pressure in this section falls below some 8 mm Hg. This phenomenon tends to prevent capillary pressure from falling to very low levels following *e.g.* precapillary constriction, at least until such constrictions are so intense that a flow limitation of capillary absorption also ensues. In other words, the increasing venous collapse automatically tends to limit the extent to which *e.g.* a reflex capillary pressure fall can result in any effective fluid absorption into the vascular bed, which is clear when the R_A and R_V curves in Fig. 1 are considered.

Determinations of effective blood viscosity in pre- and postcapillary sections

In principle the same technique as earlier used (Djojosugito *et al.* 1970) was applied for the present determination of *in vivo* viscosity in the pre- and postcapillary vascular circuits; the flow resistances for "blood" and a Newtonian fluid in the two sections were compared, at identical vascular dimensions. The latter qualification was fulfilled by keeping

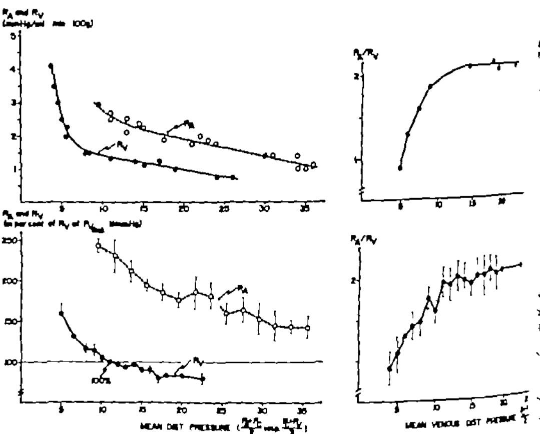


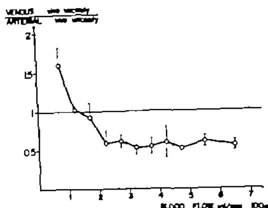
Fig. 1 The effect of varying transmural distending pressure on the precapillary (R_A) and postcap- (R_V) resistances (left), and the influence of venous transmural pressure on the pre/post-capillary resist ratio (right). The upper records are from one experiment, the lower diagrams show compiled data (m ± SE) from 9 cats.

(volume) change. By comparing R_A and R_V during blood (or erythrocyte suspension) and perfusion the Newtonian perfusate at identical distending pressures (and hence vessel geometry) the effective blo viscosity in the pre- and postcapillary vascular compartments could be determined.

Results

1 Analysis of pressure-passive changes of flow resistance in pre- and postcapillary vessels

While the transmural distending pressures were varied over a wide range, the flow resist- ances in the pre- and postcapillary vascular sections were determined from the pressure ba- and flow relation during perfusion with Newtonian fluids, i.e. for precapillary resistan ($P_A - P_C$/flow), and for postcapillary resistance ($P_C - P_V$/flow). The capillary pressure (P_C) was determined, as described above, from the prevailing rate of filtration or absorpti and the CFC value, which data allowed for calculations of the deviation of the actual P_C from the isogravimetric P_C level (P_{C_i}). The relation between transmural pressure and re- sistance in the pre- and postcapillary sections is shown in Fig. 1 both as average value from 9 compiled expts. and data from one representative experiment. As seen, precapillar resistance increases in an essentially linear fashion upon reduction of the transmural pressure



4. The quotient between postcapillary precapillary viscosity at various flow. The data are classed according to each symbol represent the mean ± S.D. that the venous viscosity exceeds the al viscosity only in the very lowest flow

he effective blood viscosity for the entire vascular bed decreases at higher flows a (e.g. Tosugito et al. 1970). Calculated effective precapillary viscosity on the other hand, does change or even increases with increasing flow i.e. if anything it alters in the opposite ction to venous and "total" blood viscosities. One consequence of these opposite nges of venous and arterial blood viscosities with changes in flow is that the ratio between ous and arterial viscosities remains below 1 over almost the whole range of flows (Fig. 4). s only hen the flow rate is around or below 1 ml/min 100 g that the theoretical pre- tion of a higher viscosity in the wide bore postcapillary vessels is true.

Results from one illustrative experiment are demonstrated in Fig. 5 in which R_A and R_V ations were analysed also at high pressures and flows. With Newtonian fluid ("plasma) rfusion RA and R decreases with increasing pressures and flows. The quotient RA/Rv o increases when the pressures are raised from low levels, however in the higher pressure nge this ratio remains largely constant throughout.

It is also seen that precapillary blood viscosity does not decrease with flow rate, at st not in the same proportion as "total" viscosity and venous viscosity: thus the ratio tween arterial and venous viscosities increases at high levels of blood flow 20 ml/min 0 g and above (not show in Fig. 4, since only fairly low flow levels were investigated in ese experiments). Consequently RA/R is considerably higher during "blood perfusion an during plasma perfusion As a result P is significantly lower blood perfusion, and articularly at higher flows where, according to Fig. 5, the presence of red cells leads to a reduction of 7 mm Hg for the same P and P levels and at a blood flow around 20 ml/ in 100 g.

Comparisons of the isovolumetric (isogravimetric) capillary pressure for Ficoll and Ficoll ith suspended erythrocytes

It is well know that together with the oxygen delivery in the capillaries a certain hyper osmolality arises within the red cells which as a result swell due to a net influx of water This means that the presence of red cells in the perfusate, which give up part of their oxygen the tissues, adds certain reabsorption force to the ordinary colloid osmotic forces. The

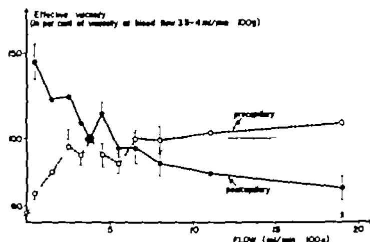
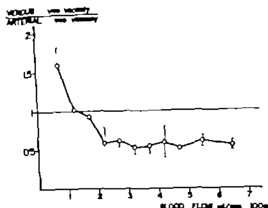


Fig. 3. Alterations of p and postcapillary re blood flow. Data fro ments, the data has b according to the flow t thus represent mean number of observatio group are given abov axis. To normalize th viscosity value at a fl 3,5 and 4 ml/min set at 100%.

mean distending pressure in the two sections as identical as possible for the p while active dimensional shifts due to smooth muscle activity were avoided by adm huge doses of vasodilator drugs. As mentioned, due to be anomalous viscosity one would *a priori* expect a higher effective blood viscosity in the widebore po vessels and therefore a fall in pre/postcapillary resistance ratio, when shifting fro substitute to "blood" perfusion. This would show up as a P_C increase when blood started, provided arterial inflow and venous outflow pressures were kept const the colloid osmotic properties of the Newtonian fluid and the "blood" were ide increased P_C should reveal itself as a *net filtration* i.e. as a net gain of volume, of the preparation.

However as it turned out quite the opposite phenomenon regularly occurred. shifting from Newtonian fluid perfusion to "blood" perfusion there was a net flu tion, indicating a P_C reduction and, consequently a relatively more pronounce of *pre*capillary than of *post*capillary resistance. This is illustrated in Fig. 2, whe fusion of the calf muscle preparation is suddenly changed from 6 Ficoll s "blood" i.e. the same 6% Ficoll solution with suspended erythrocytes to a hem 39%. Despite constant P_A and P_V levels and the same colloid osmotic pressure, t "blood" leads to a clearcut net absorption of tissue fluid i.e. to a P_C reduction. absorption rate and the CFC value, determined for blood" later in the exper actual P_C fall was calculated as approximately 3 mm Hg at this low flow level.

The phenomenon shown in Fig. 2, which was always observed when shifting fr tonian perfusion to "blood" perfusion, implies that effective blood viscosity w higher in precapillary than in the postcapillary vessels, which is contrary to wh theoretically predicted. The effective blood" viscosity in pre- and postcapilla was similarly determined in 8 experiments at varying blood" flows and the data su in Fig. 3 To normalize the data, which differed considerably between experimen different hematocrits and viscosities of the suspension medium, the values for diff levels are expressed as per cent of the viscosity value at a flow between 3.5 and 4 100 g. It is seen that effective venous viscosity decreases with increasing "blood



4. The quotient between postcapillary precapillary viscosity at various flow . The data are classed according to each symbol represent the mean ± SD that the venous viscosity exceeds the al viscosity only in the very lowest flow

the effective blood iscosity for the entire vascular bed decreases at higher flows a (e.g. ojonugko et al. 1970). Calculated effective precapillary viscosity on the other hand, does t change or even increases with increasing flow i.e. if anything it alters in the opposite ection to "venous and "total" blood viscosities. One consequence of these opposite anges of enous and arterial blood iscosities with changes in flow is that the ratio between nous and arterial viscosities remains below 1 over almost the whole range of flows (Fig. 4). is only when the flow rate is around or below 1 ml/min 100 g that the theoretical pre ction of a higher viscosity in the wide bore postcapillary vessels is true.

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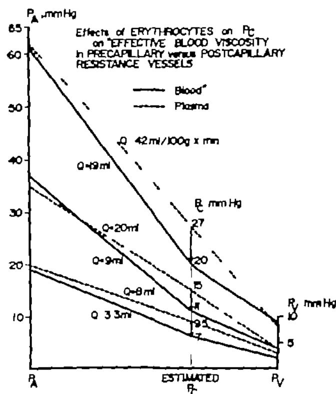


Fig. 5

	P_A	P_V	P_C	Flow	R_A	R_V	R_A/R_V	$Visc_{tot}$	$Visc_A$	$Visc_V$	$Visc_A/Visc_V$
"Plasma (Ficoll 10%)	20	3	9.5	8	1.3	0.8	1.63	1.0	1.0	1.0	1
	35	4	15	20	1.0	0.55	1.82	1.0	1.0	1.0	1
	62	8	27	42	0.83	0.45	1.84	1.0	1.0	1.0	1
"Blood" (Ficoll 10% + erythrocytes to Hct 40%)	19	2	7	3.3	3.65	1.5	2.45	2.45	2.8	1.9	1.5
	37	4	11	9.0	2.9	0.78	3.70	2.35	2.9	1.42	2.05
	61	9	20	19.0	2.15	0.58	3.70	2.10	2.6	1.28	2.05

Data from one experiment presented both as simplified pressure drop prof les along the vascular bed and a tabular form. At different pressures (P_A P_V, P_C in mm Hg) and flows (in ml/min 100 g), Newton fluid and cell suspension perfusions are compared with respect to thei effects on pre- and postcapill resistances (R_A R_V), on "total" viscosity ($Visc_{tot}$) and on pre- nd postcapillary iscosities ($Visc_A$, $Visc_V$) in ... Viscosity of the Newtonia plasma substitute is set at 1.0

importance of this reabsorption force, exerted by the red cells, has been discussed from quantitative point of view by Tybjerg Hansen (1961). This mechanism is, according t Tybjerg Hansen s calculations, capable of transporting large amounts of fluid from the tissues to the lungs. However during normal "resting" situation in skeletal muscle, the reabsorption force can be calculated to correspond to less than 1 mm Hg transcapillar pressure difference, but evidently this figure must increase considerably during increased blood flow and tissue oxygen uptake. At any rate, such an osmotic "Tybjerg Hansen effect" of red cells must be considered in the present studies. The change from filtration to absorp tion when shifting from Ficoll perfusion to Ficoll + erythrocyte perfusion (cf Fig. 2) ma thus, to an unknown extent, be due to a "Tybjerg Hansen effect" and not only to a decrease of hydrostatic capillary pressure. The capillary pressure may thus in fact be higher than pr

d from the rate of absorption and the calculated value for pre/postcapillary resistance consequently erroneously high.

order to confirm experimentally in an *in vivo* preparation the existence of such an otic reabsorption force, exerted by the red cells, and to get a rough idea of its magnitude he present experimental set-up, the P_{0_i}-values for Ficoll and Ficoll + erythrocytes carefully and repeatedly determined in 4 animals. It was then found that the average was 0.63 ± 0.07 mm Hg higher when erythrocytes were added to the Ficoll passing the ular bed of the resting calf muscles. In other words, to establish a complete balance een filtration and absorption forces, P must be 0.63 mm Hg higher in the presence of cells, implying that the red cells exerted an absorption force of this magnitude. Thus, e calculated absorption force of 3 mm Hg, required to produce the rate of fluid absorp- tion in Fig. 2, only 0.63 mm Hg, or around one fifth, can be ascribed to a "Tybjerg sen phenomenon In the present study with low flows and low O_2-consumption in ing skeletal muscle the "Tybjerg Hansen phenomenon therefore does not seem to play mportant role, and this factor was consistently disregarded in the calculations of R_A, etc, during "blood" perfusion. As will be discussed below the situation must be different ing e.g. intense muscle exercise.

Discussion

postcapillary flow resistance is one important factor determining the capillary pressure d and hence transcapillary fluid movements and plasma volume. Few attempts have, never been made to analyze how this postcapillary resistance is controlled. It has quite erally been taken for granted that active shifts in venous vessel tone constitute the most ortant factor influencing the flow resistance in this section (e.g. Hadjiminas and Öberg 9). However some scattered observations from earlier studies suggest that also other chanisms are capable of affecting the resistance in the venous section. Thus, the remarkable utoregulation of the capillary pressure when arterial pressure falls (*cf.* Folkow and Öberg 5; Öberg 1964 Järhult and Mellander 1974), can hardly be explained by an auto- ulatory reduction of the precapillary resistance alone but seems to require also an crease in postcapillary resistance. In the present series of experiments two passive enomena ha e been analysed which might reasonably be expected to affect the post- pillary resistance, namely pressure-passive alterations of vessel geometry and post- pillary blood viscosity

The results of these studies ha e shown that, as expected, the flow resistance increases in th pre and postcapillary sections when transmural distending pressure falls, provided active" adjustments of smooth muscle tone are eliminated by maintaining the vascular d maximally dilated throughout. This increase of flow resistance in both the pre and stcapillary sections, following e.g. stepwise reductions of arterial inflow pressure, occurs ughly in parallel so that no drastic shifts in the ratio between the pre- and postcapillary sistances occur as long as the average venous transmural pressure does not fall below 5 mm Hg. Below this pressure level enous resistance increases out of proportion to a rther decrease in distending pressure. This ery marked increase of postcapillary resist-

ance, which in all probability depends on an increasing degree of venous collapse (
Öberg 1967), leads to a decrease of the pre/postcapillary resistance ratio (R_A/R_V). T
ratio, which at higher pressure levels amounts to approximately 2/1 in the maximally di
muscle bed when perfused with an oxygenated Newtonian fluid, falls towards 1/1 the c
the venous transmural pressure falls below 6–8 mm Hg. Thus R_A/R_V reduction cons
one inherent passive mechanism which prevents the capillary pressure from falling u
low levels along with *e g* a reduction of the arterial pressure.

These pressure-passive changes of R_A and R_V illustrate the uncertainties inherent
determinations of R_A/R_V and P_C, at least when the original method of Pappenheimer
Soto-Rivera (1948) is employed, implying a simultaneous increase of P_V and decrease
P_A with maintenance of an isogravimetric state. It is evident that these manoeuvres
cause an increase of R_A/R_V *i.e* a change of the variable one wishes to measure.
disturbance becomes particularly marked if the procedures are performed at low ve
transmural pressures. The inherent error in the determination of R_A/R_V and P_{C_i}
considerably reduced in the present modification of the technique of Pappenheimer
Soto-Rivera, where both P_A and P_V and hence R_A and R_V are changed in the same
tion and only to a small extent, thus tending to minimize the unavoidable, experimen
induced shifts in R_A/R_V

The background to the unexpected finding of a higher "effective" blood viscosity in
precapillary than in the postcapillary vessels is not fully clear Theoretically the re
would be expected since effective viscosity of anomalous fluids, like blood, is lower
smaller the tube radius and the greater the linear flow rate. A possible explanation m
follows. The proximal, quite narrow sections of the true capillaries must be consid
part of the *precapillary* resistance section (as defined by Eliassen *et al* 1974) because
result of the increasing porosity of the capillary membrane towards the wider distal sec
and in the venules (*e.g* Zweifach and Intaglietta 1969), they are placed proximal to
equilibrium point for the filtration—absorption process along the exchange vessels.
furthermore known that a certain deformation of the red cells occurs when they are sq
ed through the metarterioles and capillaries which in proximal parts have diameters of
4–5 μm in skeletal muscle (*e g* Eriksson and Myrhage, 1972) Thus a certain force
quired to cause this "bending" of the cells (*cf* Branisch, 1971), and this will take place
cularly in the proximal end of the capillaries, while the wider down-stream exchange sec
hardly call for any erythrocyte bending or friction against the wall. Even if the force n
to deform erythrocytes is relatively small, it will nevertheless imply an energy loss
therefore an added pressure fall in these narrow metarteriolar–proximal capillary sec
The higher the blood flow and hence the more red cells pass these proximal capillary
tions per unit time, the more pronounced the regional pressure fall as a result of this "
flow"

This latter feature showed up in the present study as an almost constant, or even sl
elevated "apparent" viscosity in precapillary vessels when flow increased, while
situation one would have expected rather a *reduction* of viscosity due to the anom
viscous properties of blood with a reduced viscosity at high shear rates. Thus, what is
measured as a "precapillary blood viscosity" is not only a genuine v but presu

the energy losses inherent in the deformation and wall friction of red cells when they through the most narrow sections of the vascular bed, i.e. the distal metarterioles and ximal parts of the capillaries. The tendency for "true" blood viscosity to decrease with easing flow rate is thus offset in the most distal segment of the precapillary vessels by ly increased energy losses. The net effect is an almost unchanged, or sometimes even ncreased effective precapillary viscosity along with increases of flow.

he functional implication of this considerable increase of precapillary flow resistance red cells are added to the perfusate was demonstrated in Fig. 5, and may be further strated by the following example. Assume in a resting muscle a P_A of 100 mg Hg, a P mm Hg and a P value around 15–20 mm Hg (Eliassen *et al.* 1974), *i.e.* the R_A/R_V-o is 5–8/1. With heavy exercise and maximal vasodilatation, the R_A/R_V-ratio would rease, according to the present study, to less than 2/1 if no erythrocytes are present. With same P and P levels, P would then rise to 35–40 mm Hg, *i.e.* by 20–25 mm Hg. This ked P rise, in combination with the high CFC value in exercising muscles (0.04–0.05), uld mean extensive filtration losses. The presence of red cells implies, however, that the R_V-ratio would decrease to only 3.5–4/1 at maximal flow values (Fig. 5). In this situa 1, with P at 100 mm Hg and P at 5 mm Hg, P would assume a value around 25–27 1 Hg, P would increase by at most some 10 mm Hg. Thus, by keeping R relatively h at maximal vasodilatation and high flows, the red cells are capable of keeping P low reby reducing the filtration losses considerably. Moreover, the red cells also help to keep fluid within the vascular system in heavily exercising muscles by the earlier mentioned ybjerg-Hansen effect. Even if the "reabsorption force" exerted by this mechanism, ust be rather small in the resting state, it must increase its potency 20–30-fold in hea ily rcising skeletal muscles since it is known that their oxygen extraction then often increases old and their blood flow 10-fold. The red cells thus contribute in two important ways the preservation of the intravascular fluid volume in g heavy exercise, by helping to unteract the fluid transfer caused by the often marked regional increases in tissue osmo-ity (Mellander *et al.* 1967, Lundvall 1972).

The idea which initiated the present study was that postcapillary blood viscosity was ely to be higher than precapillary blood viscosity since *in vitro* studies have made it clear at blood viscosity increases with increases of tube dimensions and with decreases in linear w rate. If applicable to the normal vascular bed, such a regional viscosity difference uld tend to decrease R_A/R_V and hence increase P_c, perhaps even to the extent that filtra-on losses might ensue at low flow rates. The experimental findings were, however, contrary these theoretical predictions. Postcapillary blood viscosity was then decidedly lower than ecapillary blood viscosity over nearly the entire flow range. Only at the very lowest flows, low some 1 ml/min 100 g (as may occur in severe shock), did postcapillary blood visco-ity approach and eventually exceed precapillary blood viscosity. In all other situations the ost important consequence of the higher precapillary blood viscosity *in vivo* seems to be lower capillary pressure and consequently help to prevent filtration losses from the cardio-ascular system.

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References

BAECKSTRÖM, P., B. FOLKOW, E. KENDRICK, B. LÖFVING and B. ÖBERG, Effects of vasoconstriction on blood viscosity in vivo. *Acta physiol. scand.* 1971. *81*. 376–384.

BRAASCH, D., Red cell deformability and capillary blood flow. *Physiol. Rev.* 1971. *51*. 679–701.

DJOJOSUGITO, A. M., B. FOLKOW, B. ÖBERG and S. WHITE, A comparison of blood viscosity measured in vitro and in a vascular bed. *Acta physiol. scand.* 1970. *78*. 70–84.

ERIKSSON, E. and R. MYRHAGE, Microvascular dimensions and blood flow in skeletal muscle. *Acta physiol. scand.* 197‥ *86*. 11–2‥

ELIASSEN, E., B. FOLKOW, S. M. HILTON, B. ÖBERG and B. RIPPE, Pressure-volume characteristics of the interstitial fluid space in the skeletal muscle of the cat. *Acta physiol. scand.* 1974. *90*. 583–593.

FOLKOW, B. and B. ÖBERG, Autoregulation and basal tone in consecutive vascular sections of the skeletal muscles in reserpine-treated cats. *Acta physiol. scand.* 1961. *53*. 105–113.

HADJIMINAS, J. and B. ÖBERG, Effects of carotid baroceptor reflexes on venous tone in skeletal muscle and intestine of the cat. *Acta physiol. scand.* 1968. *72*. 518–532.

JÄRHULT, J. and S. MELLANDER, Autoregulation of capillary hydrostatic pressure in skeletal muscle during regional arterial hypo- and hypertension. *Acta physiol. scand.* 1974. *91*. 32–41.

KJELLMER, I., The effect of exercise on the vascular bed of skeletal muscle. *Acta physiol. scand.* 19‥ 18–30.

LUNDVALL, J., Tissue hyperosmolality as a mediator of vasodilatation and transcapillary fluid flux in exercising skeletal muscle. *Acta physiol. scand.* 1972. Suppl. 379.

MELLANDER, S., B. JOHANSSON, S. GRAY, O. JONSSON, J. LUNDVALL and B. LJUNG, The effects of hyperosmolality on intact and isolated vascular smooth muscle. Possible role in exercise hyperemia. *Angiologica* 1967. *4*. 310–322.

ÖBERG, B., Effects of cardiovascular reflexes on net capillary fluid transfer. *Acta physiol. scand.* 19‥ Suppl. ‥9.

ÖBERG, B., The relationship between active constriction and passive recoil of the veins at various distending pressures. *Acta physiol. scand.* 1967. *71*. 233–247.

ÖBERG, B., Aspects on the control of the capillary filtration process. *Cardiologica* 1974. Suppl. ‥

PAPPENHEIMER, J. R. and A. SOTO-RIVERA, Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hindlimbs of cats and dogs. *Amer. J. Physiol.* 1948. *152*. 471–491.

TYBERG HANSEN, A., Osmotic pressure effects on the red blood cells. Possible physiological significance. *Nature* (Lond.) 1961. *190*. 504–508.

WHITTAKER, S. R. F. and F. R. WINTON, The apparent viscosity of blood flowing in the isolated hindlimb of the dog and its variation with corpuscular concentration. *J. Physiol.* (Lond.), 1933. *78*. 339–‥

ZWEIFACH, B. W. and M. INTAGLIETTA, Mechanics of fluid movement across single capillaries in the rabbit. *Microvasc. Res.* 1969. *1*. 83–101.

Acta physiol. scand. 1975. 95. 191–200

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Kinetics of the Glomerular Ultrafiltration in the Rat Kidney A Theoretical Study

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Abstract

Källskog, Ö. L. O. Lindbom, H. R. Ulfendahl and M. Wolgast *Kinetics of the glomerular ultrafiltration in the rat kidney A theoretical study* Acta physiol. scand. 1975 95 191–200.

The glomerular filtration process was evaluated theoretically from micropuncture data obtained from Sprague-Dawley rats. The hydrostatic pressures in the glomerular capillaries and Bowman's space minus oncotic pressure in systemic plasma gave the net driving force at the proximal end of the glomerular capillary. From the single nephron filtration fraction the mean net driving force over the glomerular membrane was calculated to be 20 mm Hg during normotension, decreasing to 12 mm Hg during perfusion pressure of 80 mm Hg. The hydraulic permeability for one glomerulus was 0.7–0.8 nl/min 100 g b.wt. mm Hg. The pressures at the distal end of the glomerular capillaries were 13 and 6 mm Hg under the above two conditions, indicating non-equilibrium of the filtration process at the end of the glomerular capillary. It is shown that the glomerular filtration rate is mainly influenced by the driving pressures. During hypotension an increased plasma flow dependency was evident. Brenner et al. found filtration equilibrium and plasma flow dependent glomerular filtration rate in a mutant Wistar rat strain. The discrepancy between their results and ours is due to the low glomerular plasma flow and hydrostatic pressures in the Wistar rats. It is concluded from our results that both pre- and postglomerular resistances may influence the glomerular filtration rate and glomerular plasma flow independently.

The kinetics of glomerular ultrafiltration have been the object of keen interest for some years, since the variables involved in the process will determine not only the fluid balance with respect to filtration but also, indirectly, the absolute tubular fluid reabsorption, at least where the proximal tubules are concerned. The circulatory factors operative in the renal vascular system thus play a cardinal part in the control of the extracellular and plasma volumes and thereby also in the control of cardiac output and systemic blood pressure.

Essentially two modes of experimental approach have been used in evaluating the glomerular filtration process. In the earliest approach the kinetics of glomerular ultrafiltration were examined by studying the transport of molecules of different sizes (Bott *et al.* 1941, Wallenius 1954, Chinard 1952, Pappenheimer *et al.* 1951). The membrane characteristics were determined, by means of the equations presented by Landis and Pappenheimer (1963).

In more recent studies by Lambert and coworkers (Lambert *et al.* 1972, Vernlory *et al.* 1[illegible] Gasse *et al* 1974) the theoretical basis of the sieving equations was re-examined (Solr 1968 Vernlory *et al* 1973) and applied to the glomerular ultrafiltration process in the kidney

A direct approach involving determinations of driving forces and fluid flow over membrane in a single glomerulus was first presented by Brenner and co-workers (1971, 1[illegible] In these studies direct punctures of the glomerular capillaries and Bowman's space [illegible] carried out on a unique mutant Wistar strain with superficially located glomeruli. In [illegible] animals the filtration fraction could also be determined from small samples of blood [illegible] tained from large welling points on the kidney surface. The results indicated a comparatively low glomerular capillary pressure and a low single glomerular plasma flow A mathem[illegible] computer based evaluation revealed that an approximate filtration equilibrium is [illegible] somewhere within the glomerular capillary tree and that the filtration is highly dependent [illegible] plasma flow

The present evaluation of the glomerular ultrafiltration process is based on data obtained from normal full-grown male Sprague Dawley rats investigated by Källskog *et al.* (1[illegible] In the latter study the occurrence, though rare, of superficially located glomeruli was [illegible] for an estimation of the hydrostatic pressure operative over the glomerular membrane [illegible] addition continuous sampling of blood from the welling points allowed calculation of [illegible] colloid osmotic pressure in the distal end of the glomerular capillaries.

Experimental data

These were taken from Källskog *et al.* (1975). The glomerular capillary pressure as obtained by [illegible] punct res was 6[illegible].6 ± 3 2 mm Hg (mean ± S.D). The pressure in Bowman's space was 16 0 ± [illegible] which was 2.5 mm Hg higher than that the proximal tubule. The hydrostatic pressure difference [illegible] glomerular membrane was thus 46.6 mm Hg. W th a 6 plasma protein concentration (Källskog [illegible] W lgast 1973) the colloid osmotic pressure of systemic plasma is 20.3 mm Hg. The net driving force [illegible] proximal end of the glomerulus was calculated to be 6.3 mm Hg.

The pressure difference calculated from the top-flow pressure and the proximal tubular pressure [illegible] cording to Gertz *et al* (1966) was 24 2 mm Hg in one of the series studied and [illegible]6.2 mm Hg in the [illegible] thus in complete accordance w th that obtained from direct punctures. During hypotension to [illegible] pressure of 80 mm Hg the pressure d fference decreased t 18.3 mm Hg.

The filtration fraction as estimated from small blood samples w thdrawn from the distal ends of efferent arterioles (well g points) was 0 27 ± 0 06 (9) during normotension, changing non-signific[illegible] to 0.[illegible]8 ± 0.03 (= 9) at 80 mm Hg

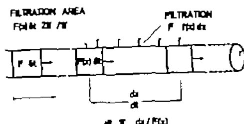
The single nephron glomerular filtration rate (SNGFR) was 14.1 ± 1 9 nl/min 100 g b.wt. (the [illegible] weighed about 300 g) under control antidiuretic cond tions, decreasing t 10.4 ± 2 4 nl/min 100 g at 80 mm Hg. The single nephron plasma flow was calculated to be 52 2 ± 7.0 nl/min 100 g and 37 1 ± [illegible] nl/min 100 g under normotensi and hypotensiv conditions, respect ely

The blood flows would be bout twice these f gures, as the sy temic hematocrit was 0.50. The mean [illegible] pressure a eraged about 1[illegible]0 mm Hg. The pressure drops over the pre- nd postglomerular [illegible] be essentially the same. The arteriolar resistance was calculated to be approximat ly 0 6 mm Hg [illegible] 100 g nl and the vascular conductance thus 1 7 l/min mm Hg 100 g b wt.

Evaluation of the glomerular ultrafiltration process

The glomerular capillaries are assumed to constitute a cylindrical tube with a constant len[illegible] x_e, and a radius r (Fig. 1). The permeability is uniform along the capillary

During a certain time interval, δt, a volume segment passes into the [illegible] llary system. W[illegible]



1. Hypothetical model of the glomerular lines assumed to constitute cylindrical with radius. The fluid filtered from the volume segment has passing from to + governed by the surface area of the segment the glomerular membrane and the time of age over the distance dx.

ring the capillary the volume of the segment is F δt, and at position x within the capillary olume is F(x) δt, F is the plasma flow at the beginning of the capillary and F(x) the at position x. The surface area of the segment facing the capillary wall is then.

rface area F(x) δt 2πr/πr² Eq 1

When passing over a distance dx a certain amount of fluid will be filtered as determined the net driving force, the permeability characteristics of the glomerular membrane and time of passage, dt.

The net driving force is in turn determined by a) the hydrostatic pressure within the merular capillary (this is assumed to be constant at all positions in the capillary bed), b) hydrostatic pressure within Bowman's space, and c) the colloid osmotic pressure of glomerular capillary blood. The latter factor can be calculated from the protein concentration with the aid of the formula given by Landis and Pappenheimer (1963)

π 2.1 C 0.16 C² + 0.09 C³ Eq 2

The protein concentration at any position within the capillary is then calculated from the concentration in arterial plasma, C_a, and the filtration fraction. The increase in filtration ction per length unit is denoted f(x), and the increase over the distance dx is thus f(x)dx. total filtration fraction produced up to position x is accordingly $\int f(\ )dx$. The protein ncentration can then be expressed as $C_a/(1 - \int_0^x f(x)dx)$. The oncotic pressure is:

$$2.1\left[\frac{C_a}{1 - \int^x f(\ )\,dx}\right] \quad 0.16\left[\frac{C_a}{1 - \int^x f(x)\,dx}\right] \quad 0.09\left[\frac{C_a}{1 - \int^x f(\ )\,dx}\right] \qquad \text{Eq. 3}$$

Fig. 2 this pressure is related to the filtration fraction for 5%, 6% and 7% protein concentrations, where 6 is the concentration found during antidiuresis in the experimental ries. In the following, Eq 3 is denoted

$$g\left(\int^x f(\ )\,dx\right)$$

The net driving force on olume segment located at x is obtained as.

Eq 4

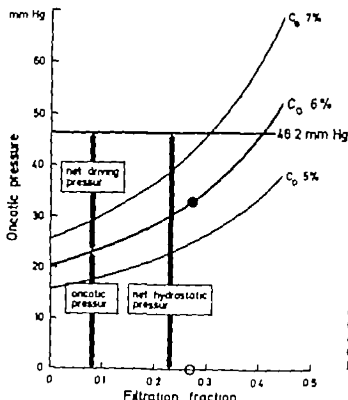


Fig. 2. Relation between filtration fraction and plasma oncotic pressure at the end of the glomerular capillaries. C_0 refers to various protein concentrations of systemic plasma. The oncotic pressure is calculated from the formulas C = C_0/(1-filtr.frac.) and π = 2.1 C + 0.16 C² + 0.009 C³. The circle gives the end-capillary oncotic pressure at a filtration fraction of 0.27 and indicates a residual net driving force of about 13 mm Hg.

where P_{glom} is the glomerular capillary pressure and P_{Bowm} the pressure in Bowman's space. The arithmetic sum of these two pressures was 46.3 mm Hg and can be seen in Fig. 2.

The passage time for the volume segment over the distance dx is calculated (see Fig. 1) by dividing the volume of the capillary system between x and x + dx by the actual flow F at position x, as follows

$$\text{Passage time} = \pi r^2 \cdot dx/F(x)$$ Eq.

The volume of fluid filtered in the segment x to x + dx is then obtained by multiplying the volume of the segment when it enters the glomerulus, F · dt, by the filtration fraction increase f(x)dx produced during the passage from x to x + dx. This volume will be determined as

$$F \cdot dt \cdot f(x)\,dx = \left[P_{glom} - P_{Bowm} - g\left(\int^x f(x)\,dx\right)\right] \cdot K \cdot \frac{2\pi r \cdot F(x)\,dt}{\pi r^2} \cdot \frac{\pi r^2 dx}{F(x)}$$

filtered volume = net driving force x permeability factor x surface area x passage time

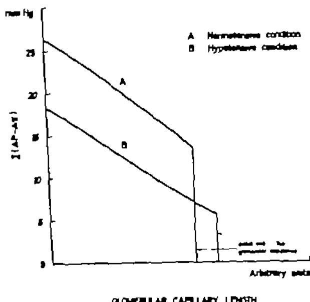
or

$$F \cdot f(x)\,dx = \left[P_{glom} - P_{Bowm} - g\left(\int^x f(x)\,dx\right)\right] \cdot K \cdot 2\pi r \cdot dx,$$ Eq.

where K is a permeability factor.

The total filtered volume up to distance x is then obtained by integrating the equation from x = 0 to x = x

$$F \int_0 f(x)\,dx = K \cdot 2\pi r \int^x \left[P_{glom} - P_{Bowm} - g\left(\int f(x)\,dx\right)\right] dx$$ Eq.



3 Relation between net ...ng pressure and glomerular ...lary length under normo- (A) hypo-(B)-tensive conditions. ... filtration equilibrium is ...ned under these conditions.

equation is easily solved graphically. For this purpose a series of filtration fractions 1 0.02, 0.03–0.27 are assumed. The driving forces valid when the filtration fractions are ,1 0.02 and so on are then calculated from Eq 2 and 3 and are shown in Fig. 2 (curve 6%). ...o integration boundaries 0–x x_1–x_2, x_2–x_3 etc. are then calculated. This is performed by ...ltiplying the mean net driving force during a particular interval by a length that will give ...constant filtration volume. The first length, where a filtration fraction of 0.01 is produced, ...placed at one length unit. During the next interval (filtration fraction increase from 0.01 0.02) the mean net driving force is somewhat smaller and the length x x_1 is propor...nately longer. The procedure is repeated until a total filtration fraction of 0.27 i.e. that ... and experimentally is reached. In Fig. 3 the relation between the glomerular capillary ...ngth (arbitrary units) and the net driving force is depicted. The upper curve represents ...ntrol antidiuretic conditions and the lower curve hypotensive conditions of about 80 mm ... The hydraulic permeability factor K_f was considered to be unchanged. The ratios be...een the flows, F under the two conditions were obtained from the formula.

$$F = \frac{\text{SNGFR}}{\text{filtration fraction}} \qquad \text{Eq 8}$$

...s seen in Fig. 3, the length of the glomerular capillaries appears t differ under the two ...nditions. This is probably not due to change in the functional length or a change in ...pillary permeability but rather to an error in determination of some of the parameters ...ployed in the calculations.

The results indicate that no equilibrium with respect to driving forces is obtained in the ...stal end of the glomerular capillary. Under normotensive conditions the net driving force ... hypotension about 6 mm Hg. The mean net driving

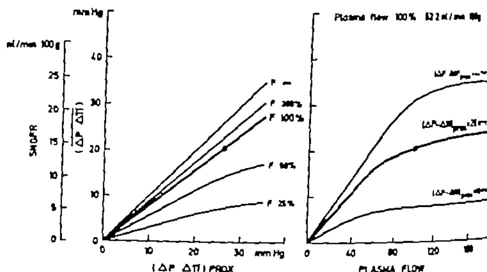


Fig. 4 The relation between the mean net driving force and the SNGFR and the driving force in the proximal end of the glomerulus (left panel) and of the single nephron plasma flow (right panel). The ... represent normal hydropenic conditions. The heavy lines represent normal flows and normal p... respectively. The figure indicates that SNGFR is pressure-dependent whereas its dependency on ... flow is less pronounced. At a plasma flow of about half the normal, the plasma flow dependency b... predominant.

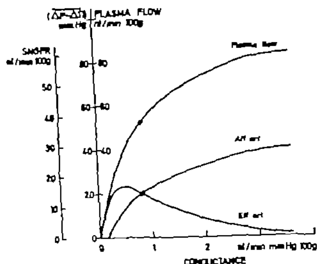
force for glomerular filtration is estimated to be 20 mm Hg during normotension, dec... to 12 mm Hg when the perfusion pressure is reduced to 80 mm Hg. This reduction i... portionately somewhat greater than the reduction in single nephron GFR which dec... from 14.1 to 10.4 nl/min and 100 g b wt. The hydraulic permeabilities are calculated 0.7 and 0.8 nl/min 100 g mm Hg under the two conditions, respectively.

From the information on the kinetics of glomerular ultrafiltration under normal diuretic conditions the dependence of the filtration on pressure and on plasma flow c... evaluated. For this purpose the permeability and functional length are assumed to ... same as under normal conditions.

Fig. 4 (left panel) shows the calculated relation between the net driving force in the ... mal end of the glomerular capillaries, on the one hand, and the mean glomerular net d... force and the SNGFR, on the other. The different curves refer to different single ne... plasma flows. Analogously the right panel in the figure shows the relation betwe... plasma flow on the one hand and the mean net driving force, and the SNGFR, on the ... for a number of assumed net driving forces in the proximal end of the glomerular capi... The heavy line represents the relation under normal pressure conditions. The black ... indicate normal antidiuretic conditions with respect to both plasma flow and net d... force. The calculations have been performed from Eq. 4.

The figure indicates that at normal plasma flows the SNGFR will be almost li... related to the net driving force at the proximal end of the capillary. Moreover a mo... increase or decrease in plasma flow will cause relatively small changes in the SNGFR.

In Fig. 5 the influence of the pre- and postglomerular conductances on the mea... driving force and the SNGFR, and also their influence on the single nephron plasma



5. The influence of pre- and postglomerular conductances on 1 the single nephron plasma flow (upper 2 the SNGFR, and 3 the mean net driving force (lower lines). The black dots represent normal conditions. The conductance was changed in one vascular segment, while the other conductance was kept unchanged. A preglomerular relaxation will lead to an increased SNGFR. A postglomerular relaxation will lead to decreased SNGFR. A maximum value is obtained when the postglomerular conductance is about of the control value. At very low postglomerular conductances the SNGFR and mean net driving force rapidly decreases towards zero

analysed (the black dots indicate normal conditions, under which the pre- and postglomerular conductances are approximately the same). This analysis involves calculation of the glomerular capillary pressure, P_{glom}, and the glomerular plasma flow F_a, as follows:

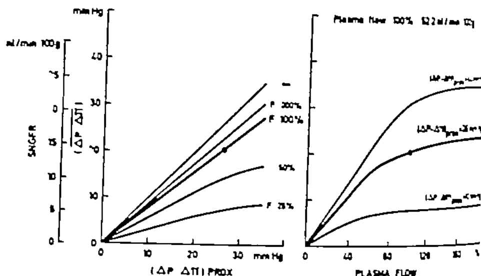
$$L_{pre\ glom}(P_{syst} - P_{glom}) = L_{post\ glom}(P_{glom} - 0) \qquad \text{Eq. 9}$$

where L are the pre- and postglomerular conductances and P_{syst} the systemic blood pressure. The pressure in Bowman's space is assumed to be proportional to the SNGFR. This assumption would seem reasonable since an increased SNGFR will cause a tubular dilatation which will reduce the resistance for tubular fluid flow), but a concomitant decrease in fractional reabsorption will have the opposite effect (Lewy and Windhager 1968). It should be pointed out that in performing the analysis the preglomerular conductance was changed while the postglomerular conductance was kept at its normal value, and vice versa.

As expected the single glomerular plasma flow increased with an increase in either the pre- or the postglomerular conductance.

Relaxation of the preglomerular vessels (aff art. in Fig. 5), implying an increased conductance, will be concomitant with an increased SNGFR as a consequence both of an increased glomerular capillary pressure and of an increased glomerular plasma flow. Reversely a decreased conductance will cause a rapid decrease in SNGFR.

A successively increased conductance of the postglomerular vessels (eff. art.) will lead to a successively decreased SNGFR, whereas a decreased conductance will cause a rise in SNGFR



F g. 4 The relation between the mean net driv ng force and the SNGFR and the driving force at the [...]mal end of the glomerulus (left panel) and of the single nephron plasma flow (right panel). The [...] represent normal hydropenic conditions. The heavy lines represent normal flows and normal pr[...] respectively The f gure indicates that SNGFR is pressure-dependent whereas its dependency on [...] flow is less pronounced. At a plasma flow of about half the normal, the plasma flow dependency is predominant.

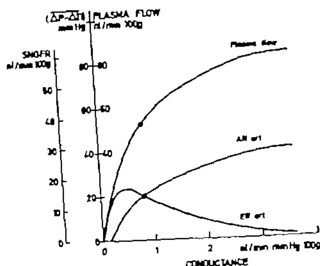
force for glomerular filtration is estimated to be 20 mm Hg during normotension, dec[...] to 12 mm Hg when the perfusion pressure is reduced to 80 mm Hg. This reduction is [...]portionately somewhat greater than the reduction in single nephron GFR which dec[...] from 14 1 to 10 4 nl/min and 100 g b.wt The hydraulic permeabilities are calculated [...] 0.7 and 0.8 nl/min 100 g mm Hg under the two conditions, respectively

From the information on the kinetics of glomerular ultrafiltration under normal diuretic conditions the dependence of the filtration on pressure and on plasma flow [...] evaluated. For this purpose the permeability and functional length are assumed to be [...] same as under normal conditions.

Fig. 4 (left panel) shows the calculated relation between the net driving force in the p[...]mal end of the glomerular capillaries, on the one hand and the mean glomerular net dr[...] force and the SNGFR on the other. The different curves refer to different single cap[...] plasma flows. Analogously the right panel in the figure shows the relation betwe[...] plasma flow on the one hand and the mean net driving force, and the SNGFR, on the o[...] for a number of assumed net driving forces in the proximal end of the glomerular cap[...] The heavy line represents the relation under normal pressure conditions. The bl[...] indicate normal antidiuretic conditions with respect to both plasma flow and net dr[...] force. The calculations have been performed from Eq 4

The figure indicates that at normal plasma flows the SNGFR will be almost li[...] related to the net driving force at the proximal end of the capillary Moreover, a ma[...] increase or decrease in plasma flow will cause relatively small changes in the SNGFR.

In Fig. 5 the influence of the pre- and postglomerular conductances on the mea[...] driving force and the SNGFR and also their influence on the single nephron plasma[...]



The influence of pre- and postglomerular conductances on 1. the single nephron plasma flow (upper 2. the SNGFR, and 3. the mean net driving force (lower lines). The black dots represent normal condi- The conductance as changed in one vascular segment, while the other conductance was kept un- ed. A preglomerular relaxation will lead to an increased SNGFR. A postglomerular relaxation will decreased SNGFR. A maximum value is obtained when the postglomerular conductance is about of the control value. At very low postglomerular conductances the SNGFR and mean net driving force ly decreases towards zero

analysed (the black dots indicate normal conditions, under which the pre and post merular conductances are approximately the same). This analysis involves calculation he glomerular capillary pressure, P_{glom} and the glomerular plasma flow F_{pl} as follows

$$L_{pre\,glom}(P_{art} \quad P_{glom}) \quad L_{post\,glom}(P_{glom} - 0) \qquad \text{Eq. 9}$$

re L are the pre and postglomerular conductances and P_{art} the systemic blood pressure. pressure in Bowman s space is assumed to be proportional to the SNGFR. This as- ption would seem reasonable since an increased SNGFR will cause a tubular dilatation ich ll reduce the resistance for tubular fluid flow), but a concomitant decrease in frac- nal reabsorption will have the opposite effect (Lewy and Windhager 1968). It should be nted out that in performing the analysis the preglomerular conductance was changed le the postglomerular conductance was kept at its normal alue, and ice versa.

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A successively increased conductance of the postglomerular vessels (eff art.) will lead to a ccessively decreased SNGFR, whereas a decreased conductance will cause a rise in SNGFR

which will reach its maximum when the conductance has decreased to about half of the original value. A further reduction will cause a rapid fall in SNGFR. This fall is then due to a concomitant decrease in single glomerular plasma flow

Discussion

The ideas obtained in this study about the kinetics of glomerular filtration contrast sharply with those obtained by Brenner *et al* (1971) in investigations on the unique mutant Wistar rat strain. In their studies the driving force in the proximal end of the glomerulus was about mm Hg. Along the glomerular capillaries the driving force was found to decrease successively reaching a value close to zero somewhere at the distal end Owing to the uncertainty as to where the driving force was zero, the filtration permeability coefficient could not be determined On the basis of their findings it is clearly evident that the glomerular filtration to be highly dependent on plasma flow—an interpretation which is supported by the present evaluation seen in Fig. 4 (the plasma flows in the Wistar strain are less than half those obtained in the present study) The hydrostatic pressure within the glomerulus will nevertheless be a determinant of the filtration rate in accordance with that predicted in Fig. 4. The influence will, however be fairly moderate since the oncotic pressure shows a steep increase (as compared with the low hydrostatic pressure) when the filtration fraction increases above the figure of 0 3 found in the Wistar strain (see Fig. 7)

Further according to the view on the dynamics of glomerular ultrafiltration presented by Brenner and coworkers the glomerular filtration rate can only be increased by a relaxation of the preglomerular vessels, as the latter will cause an increase in both the glomerular capillary pressure and the plasma flow (Fig. 4) An increase in the postglomerular resistance will not cause an increase in filtration rate, since the effect of a higher hydrostatic pressure within the glomerulus will be counteracted by the decreased plasma flow

The discrepancy between the evaluation made in the present study and that made by Brennet *et al* is thus not only quantitative but also qualitative. This is explained by differences in the values for both glomerular plasma flow (52 versus 20 nl/min 100 g) and glomerular capillary hydrostatic pressure (63 versus 45 mm Hg) The most interesting finding in the present investigation is that a filtration equilibrium is not attained, and that the filtration can thus be controlled by both the pre and postglomerular resistances and can vary with changes in plasma flow This means that the glomerular filtration rate can remain constant despite changes in plasma flow as has been demonstrated experimentally (Kill *et al* 19 and Pitts 1968)

If however the glomerular plasma flow in the mutant Wistar rat is increased to about 50 nl/min 100 g—*i e* about the same value as presented in this paper (by iso-oncotic plasma expansion)—a state of filtration non-equilibrium will be achieved (Deen *et al* 1973). In this case the mean net driving force could be calculated to be about 4–6 mm Hg, thus considerably lower than that reported here The hydraulic permeability will accordingly be estimated to be about 0 08 nl/s mm Hg, which would mean some 1 6 nl/min and 100 g b wt. *i.e* about twice the figure obtained in the present study

Using the Poiseuille formula and assuming a pore radius of 50 Å and a viscosity of

filtrate of 0.01 poise (Landis and Pappenheimer 1963), the permeability factor $A_p/\Delta x$ (pore area over pore length per glomerulus) is calculated at 3 cm/100 g rat, thus in a 300 g rat, will be 9 cm.

The determination will not permit calculation of the pore area, A_p. Assuming a pore length of 0.3 μm, however the area per glomerulus of a 300 g rat will be 2.71 10^{-4} cm² giving a total surface area of 19 10^{-4} cm² as found by Kirkman and Stowell (1942), the fractional pore area is estimated to be 14%. In the dog kidney Gassee *et al* (1974) estimated the factor $A_p/\Delta x$, by the sieving of fractions of polyvinylpyrrolidine, to be 1.35 10^{6} cm per kidney If the corresponding factor of 9 cm for one glomerulus, found here is multiplied by 30 000, *i.e.* the number of glomeruli in a rat kidney and assuming that the in vivo kidney weight is about 1 7 g, the permeability per gram kidney is calculated to be 1 6 10^{5} cm in reasonable accordance with that of Gassee *et al.* These $A_p/\Delta x$ values are considerably larger than those in capillary beds of other organs, but are similar to those obtained for peritubular capillaries. The driving force for the fluid reabsorption has been estimated in this department to be about 14 mm Hg (Wunderlich *et al* 1971 Wolgast *et al.* 1973 Källskog and Wolgast 1973). The more precise calculation of the present study gives a net driving force of 11 mm Hg, *i.e.* about half the driving force valid for glomerular ultrafiltration. As the transmembranal fluid flow is about the same as over the glomerular membrane, the permeability factor $A_p/\Delta x$, per gram kidney weight will be some 3 10^{5} cm. The total area of the peritubular capillaries is, however considerably larger *i.e.* about 350 cm² per gram kidney (Kugelgen and Braunger 1962). Assuming the same pore length of 0.3 μm as for glomerular capillaries (the pore length is probably less than that in the glomerular membrane as the latter will consist of both the capillary and tubular wall), the fractional pore area will be about 2

References

P A and A N RICHARDS, Passage of protein molecules through glomerular membranes. *J Biol Chem* 1941 *141* 791

BRENNER, B M J L TROY and T M. DAUGHARTY The dynamics of glomerular ultrafiltration in the rat *J clin Invest* 1971 *50* 1776–1780

BRENNER, B M W M DEEN, and C. R ROBERTSON, *The physiological basis of glomerular ultrafiltration in kidney and urinary tract physiology* Ed U Thurau, London 1974 336

PAPPENHEIMER, F P Possible mechanisms of formation of glomerular fluid I *Transactions of the Third Josiah Macy Jr Conference on Renal Function* (New York, Josiah Macy Foundation 1952).

DEEN, W M J L TROY C R ROBERTSON and B. M BRENNER, Dynamics of glomerular ultrafiltration in the rat IV Determination of the ultrafiltration coefficient *J clin Invest* 1973, *52*, 1500.

GASSEE, J P P DECOODT A VERNIORY and P P LAMBERT Autoregulation of effective glomerular filtration pressure *Amer J Physiol* 1974, *226*3 616–623.

GERTZ, K H J A MANGOS, G BRAUN and H D. PAGEL, Pressure in the glomerular capillaries of the rat kidney and its relation to arterial blood pressure. *Pflügers Arch. ges Physiol* 1966, *288* 369–374.

KÄLLSKOG, Ö and M WOLGAST Driving forces over the peritubular capillary membrane in the rat kidney during antidiuresis and saline expansion. *Acta physiol scand.* 1973, *89* 116–125.

KÄLLSKOG, Ö L O LINDBOM, H R ULFENDAHL and M. WOLGAST Kinetics of the glomerular ultrafiltration in the rat kidney An experimental study *Acta physiol scand.* 1975 In press.

KALLSKOG, F J KILLIAN and E LÖVING, Role of autoregulation in maintaining glomerular filtration rate at large urine flow *Acta physiol scand* 1969, 76 4–39.

KIRKMAN, H and R E STOWELL, Renal filtration surface in the albino rat. *Anat Rec* 1942, *82*, 373–384.

KÜGELGEN, A and B. BRAUNGER Quantitativ Untersuchungen über kapillaren und Tubuli der Hundeniere. *Z Zellforsch.* 1962, *214*, 764–778

LAMBERT P P A. VERNIORY J P GASSEE and P FICHERROULLE, Sieving equations and effective filtration pressure. *Kidney Intern.* 197., 2 131 146.

LANDIS, E. M. and J R. PAPPENHEIMER, Exchange of substances through the capillary all. Handb physiology Circulation II Waverly Press, USA. 1963. 961 1034.

LEWY J E. and E. E. WINDHAGER, Peritubular control of proximal fluid reabsorption in the rat k. *Amer J Physiol* 1968 *214* 943–954

PAPPENHEIMER, J R., E. M RENKIN and L. BORRERO, Filtration diffusion and molecular sieving th peripheral capillary membranes. A contribution to the pore theory of capillary permeability. *Am Physiol.* 1951 *167* 13–46.

PITTS, R. F *Physiology of the kidney and body fluids.* Chicago, Year Book Medical Publ. 1968.

WALLENIUS, G Renal clearance of dextran as a measure of glomerular permeability *Acta Soc upsalien* 1954. Suppl 4

SCHNERMANN J Physical forces and transtubular movement of solutes and water In *Kidney and tract physiology* Physiology series 1 vol. 6. Ed. K. Thurau, Butterworths, London, 1974 157-

SOLOMON A K Characterization of biological membranes by equivalent pores *J gen. Physiol* 1968 *51* 335–364

VERNIORY A., R DU BOIS, P DECOODT J P GASSEE and P P LAMBERT The measurement of the permeability of biological membranes. Application to the glomerular wall *J Gen. Physiol.* 1973. 62.

WOLGAST M E. PERSSON, J SCHNERMANN, H ULFENDAHL and P WUNDERLICH, Colloid osmotic pressure of the subcapsular interstitial fluid of rat kidneys during hydropenia and volume expansion. *Pflügers Arch. ges. Physiol.* 1973. *340*. 123–131

WUNDERLICH, P E. PERSSON J SCHNERMANN, H ULFENDAHL and M. WOLGAST Hydrostatic pressure in the subcapsular interstitial space of rat and dog kidneys. *Pflügers Arch. ges. Physiol.* 1971 *328*.

Acta physiol. scand. 1975, 95, 201–202

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The Effects of Hypothermia on Submaximal and Maximal Work Performance

By

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Keywords: Hypothermia, exercise, heart rate, atropine, β blockade, aerobic power output

... ell known that hypothermia affects physical performance (Keatinge 1969) and may ... lt in death if exposure is severe (Pugh 1964). It has also been shown that swimming in ... ater (18°C) results in an increased metabolic rate at a given speed and a decreased ... rt rate at a given oxygen uptake (Nielsen 1973, Nadel *et al.* 1974, Holmér and Bergh ...). Similar results have been obtained during submaximal bicycle exercise (Nadel *et al.* ...), but to our knowledge no attempts have been made to explain this HR response to ... othermia. We also intended to study the effect of hypothermia on the response to maxi... exercise under standardized laboratory conditions.

... the present study we made 5 normal healthy male subjects hypothermic by allowing ... n to swim at a speed of 0.55 m/s (V_{O_2} about 50% of V_{O_2} max) for 30 min (until rectal ... perature of approximately 35°C was reached) in a swimming flume (Åstrand and Engles... 1972) containing water at 18 C. Immediately after the end of the swim the response to ... maximal and maximal exercise on an arm and leg bicycle ergometer under standardized ... oratory conditions with and without atropine (2 mg i.v.) was measured and the data ... lected were compared to results obtained during normal cycling. In addition one subject ... s also studied after β-blockade with propranolol (10 mg i.v.).

The results show that following hypothermia V and V_{O_2} at a given submaximal work ... d of 100 W increased (p 0.05, Table I) whereas the heart rate was reduced by 14 beats/... n. Atropine reversed this latter change but was without effect on the observed increase ... the metabolic cost of work. The effect of atropine on the heart rate was of the same order ... magnitude as reported earlier from studies in normothermic conditions (Ekblom *et al.* ...77). In contrast the administration of propranolol did not further reduce HR during ... bmaximal exercise in hypothermia. At maximal effort hypothermia significantly reduced ... max, V_{O_2} max and HR max by .5 l/min, 0.42 l/min and 26 beats/min respectively. These ... anges were unaffected by atropine (Table I). These data show that hypothermia has a ... rofound effect on physical performance by reducing maximal aerobic power whilst in... reasing the submaximal cost, placing greater relative strain on the individual. The change ... V_{O_2} max was related to the decrease in cardiac frequency and this relationship was un...

On leave of absence from the Medical Research Council, Environmental Physiology Unit.

TABLE I

	V_E BTPS l/min	V_{O_2} STPD l/min	V_{CO_2} STPD l/min	RQ	HR beats/min
(a) *Submaximal responses to exercise at a gi en ork load f 100 W*					
Normothermia n = 5	41 1±7 1	1.52±0.09	1 43±0.18	0.94±0 14	[illegible]
Hypothermia n = 5	64.1 ±17.8	1 90 ±0.21	1 79 ±0.29	1.00±0 09	[illegible]
Hypothermia (plus Atropine) n = 5	64.5±15.5	1 90±0.18	1 97±0.30	1.04±0.11	[illegible]
(b) *Maximal esponses to exercise*					
Normothermia n = 5	165.0±9 5	3 87±0.30	4 40±0.32	1 13±0.02	[illegible]
Hypothermia n = 5	145.3±16.4	3 45 ±0.33	4.20±0.35	1.2±0 12	[illegible]
Hypothermia (plus Atropine) n = 5	140.2±10.9	3 44±0.38	3.99±0.41	1 16±0 04	[illegible]

Significance Normothermia–hypothermia $P < 0.001$ $P < 0.01$ $P < 0.05$
Hypothermia–hypothermia (plus Atropine) ++$P < 0.001$ ++$P < 0.01$ +$P < 0.05$

affected by atropine. The results from this study suggest that hypothermia may b[e] direct effect on the cardiac muscle and possibly that the β-receptor activity is reduced hypothermia.

References

ÅSTRAND, P.-O and ENGLESSON, A swimming flume. *J appl Physiol* 1972, 33 514.

EKBLOM, B., A. GOLDBARG, Å KILBOM and P-O ÅSTRAND Effects of atropine and propranolol on oxygen transport ystem during exercise n man. *Scand J clin. Lab Invest* 1972, 30, 35-4...

HOLMÉR, I nd U Bergh, Metabolic and thermal response to swimming in water at arying temper... *J appl Physiol* 1974 37 702 705

KEATINGE, W R *Surviv l in old ter* 1969 Oxfo d, England. Blackwell.

NADEL, E. R., I HOLMÉR, U BERGH, P-O ÅSTRAND nd J A J STOLWIJK, Energy exchanges of swimming man. *J appl. Ph siol.* 1974 36. 465–471

NIELSEN B., Metabolic reactions to cold duri g swimming at different speeds. *Arch Sci. Ph*... 27 207–211

PUGH L. G C., Deaths from exposure on Four I ns W lking Compet tion, M rch 14-15, 1964 La... 1964 1210-1 1...

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From August Krogh Institute, University of Copenhagen, Denmark

Capillary Density in Skeletal Muscle of Man

By

PER ANDERSEN

the basic work on skeletal muscle capillaries by *Krogh (1918–19)*, the capillary density ammalian muscle has been described both with respect to red and white muscles and aining. Similar studies on man are very few due to methodological limitations.

the present study a new method has been used making it possible to describe capillary ty and number of capillaries around different fibre types in human skeletal muscle.

le biopsy samples from the lateral portion of the quadriceps femoris muscle were taken from 9 healthy e males. 5 subjects were untrained (group A, $\dot{V}_{O_2}$ max = 46 ml/kg min (41 53)), and 4 subjects were nace-trained (group B, $\dot{V}_{O_2}$max 63 ml/kg min (58–73)). Three of the untrained subjects (group C) pated in training program 4 times week for about 7 weeks, pedalling bicycle ergometer for 20 *work intensity requiring approximately 80 of $\dot{V}_{O_2}$max. This resulted in 13% (9–20) increase* ₂max.

e muscle samples were mounted in an embedding medium and frozen in isopentane cooled to freez-point. Serial transverse sections (10 μm thick) were cut in cryostat at 20°C. For fibre classification pe I, IIA, IIB, and IIC sections were stained for *myofibrillar ATP-ase, preincubated at various pH* oke and Kaiser 1970). Sections to be stained for capillaries were kept at 70°C.

ning procedure. Bring sections to room temperature; fix in Carnoy fixativ (10 min, 21°C), rinse eionized after digest glycogen with 1 α-amylase (Sigma A-6755; 30 min, 37°C), rinse in redistilled er, oxidize in 1 periodic acid (HIO_4, *10 min, 21°C*), stain with *Schiff* reagent, rinse in water de-rate, clear and mount. This method (Amylase-PAS) stains most likely mucopolysaccharide in the cle and capillary basement membrane (Plate I). The staining was photographed and copies produced X enlargement). An area (A) was framed on the photograph and capillaries were confirmed by direct roscopy. Fibre types within A were plotted on the photograph. Fibre area was measured by the grid hod (Edström and Torlegård 1968, 69). The number of fibres of each type used for measuring area ended on the fibre composition within A (mean and range: Type I 38 (20–60), type IIA: 27 (9–59) IIB 14 (3–22) and type IIC 3 (1–6)). The area data ere not accepted unless the total area calculated m mean area and number of each fibre type within A agreed to within ±5 of the actual measured of A. Because of the low number of type IIC fibres, these alues ll not be discussed.

ean values, *SE and range for the whole group* (9) are given in *Table I. Two* subjects om group B had no type IIB fibres (n 7).

The mean capillary density of 317±15 cap./mm correspoods well with the electron-icroscope study (*Saltin et al. 1968*), but is lower than light-microscope data (Hermansen nd Wachtlova 1971). The number of capillaries around type I fibres was not different from e type IIA fibres, both were higher than the number around type IIB fibres (p 0.01). ibre areas (type I 4 980 350 ($\mu m)^2$ type II 5 500 ± 330 ($\mu m)^2$) are within the range ported in the literature (see Gollnick *et al.* 1972). Type IIA area was greater than either pe I area or type IIB area (p 0.01). There was no difference between mean fibre area f type I and IIB.

TABLE I. Mean values, SE and range for measured and calculated muscle characteristics a 3, r n = 6( ).

	V_{O_2} max ml/kg min	A mm²	Fibre composition in area A					Capillaries per mm²	Capillaries per fibre
			I	% IIA	IIB	IIC	No. of fibres		
Mean	54	0.593	59	29	11	1.5	113	317	1 [illegible]
S.E.	4	0 090	6	4	2	0.7	14	15	[illegible]
Range	41	0 284	29	15	0	0	61	259	[illegible]
	75	1 116	82	58	21	6.3	180	390	2 [illegible]

When the mean number of capillaries around a fibre type was expressed relative to mean area for that fibre type, no difference was found between type IIA and IIB, but were lower (p < 0 01) than type I These data are reciprocally proportional to the diffusion distance described by Krogh (1918/1919) and are compatible with the biochemical and contractile differences between the type I and II fibres (Essén *et al* 1975). However the data did not reflect the biochemical differences between type IIA and IIB fibres. type I fibres have more capillaries than type II fibres, capillary density varies with composition (group A type I 45% ±8 of total area, 48 % ±8 of total number 3[illegible] cap./mm group B type I 71 ±5 of total area, 72% ±4 of total number 355 17 mm (group A vs. B p 0 01)).

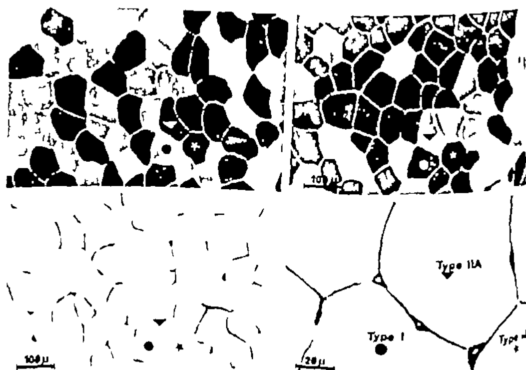


Plate I Photomicrographs of serial sections Upper left and right ATP-ase staining, preincubated at pH 10.3 and 4 6, respectively (86X). Lower left and right Amylase-PAS staining (86 and 430X).

of capillaries type			Fibre area (μm)² type				Number of capillaries around fibre type per fibre type area 10^{-3} (μm)$^{-2}$			
IIA	IIB	IIC*	I	IIA	IIB	IIC*	I	IIA	IIB	IIC*
4.5	3.5	4.5	4 980	5 770	4 570	3 900	1.00	0.79	0.76	1.26
0.3	0.4	0.7	350	340	460	540	0.03	0.02	0.03	0.25
3.0	2.0	3.0	3 320	3 670	2 960	1 640	0.90	0.71	0.63	0.77
5.8	5.2	7.0	6 200	6 780	6 360	5 260	1.14	0.86	0.84	2.44

group C fibre composition was the same before and after training (type I respectively 13 and 51 ± 7 of total area), but the capillary density increased with training from 15 cap/mm to 356 ± 23 cap/mm ($p < 0.05$). The capillary/fibre ratio varied also the training state (group A, 1.30 ± 0.13 group B 2.15 ± 0.12 ($p < 0.01$) group C be and after training 1.39 ± 0.18 and 1.95 ± 0.06 (p 0.05)). The difference was not only to a different mean fibre area (group A, 4 500 ± 374 (μm)² group B 6 050 ± 60 (μm)² 0.05); group C no significant change with training) (Hermansen and Wachtlova 1971), the difference in fibre composition and capillary density did affect the value.

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References

…, M. H. and K. K. Kaiser, Three myosin ATPase systems: The nature of their pH lability and …hydryl dependence. *J. Histochem. Cytochem.* 1970, *18* 670–672.

…, L. and K. Tornqvist, Area estimation of transversely sectioned muscle fibres. *Z. wiss. Mikr* … 69, 69 166–178.

…, B., E. J. …, J. Henriksson, A. W. Taylor and B. Saltin, Metabolic characteristics of fibre …pes in human skeletal muscles. *Acta physiol. scand.* 1975. 95 153–165

…, P. D., R. B. Armstrong, C. W. Saubert IV, K. Piehl and B. Saltin, Enzyme activity and fiber …position in skeletal muscle of untrained and trained men. *J. appl. Physiol.* 1972. 33, 31 319.

…, L. and M. Wachtlova, Capillary density of skeletal muscle in well-trained and untrained … *J. appl. Physiol.* 1971 30, 860–861.

…, A., The number and distribution of capillaries in muscles with calculations of the oxygen pressure … necessary for supplying the tissue. *J. Physiol. (Lond.)* 1918/1919. 52, 409–415

…, B. … Blomqvist, J. H. Mitchell, R. L. Johnson, J. K. Wildenthal, and C. B. Chapman, …response … exercise after bed rest and training. *Circulation* 1968, Suppl. 7

TABLE I Mean values, SE and range for measured and calculated muscle characteristics i t,r n = 6( ).

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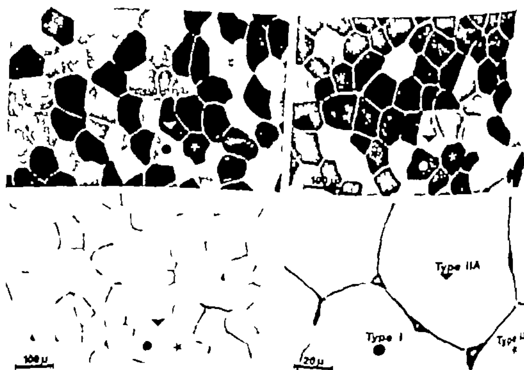


Plate I. Photomicrographs of serial sections. Upper left and right ATP-ase staining, preincubated at 10.3 and 4.6, respectively (86X). Lower left and right. Amylase Pas staining (86X and 430X).

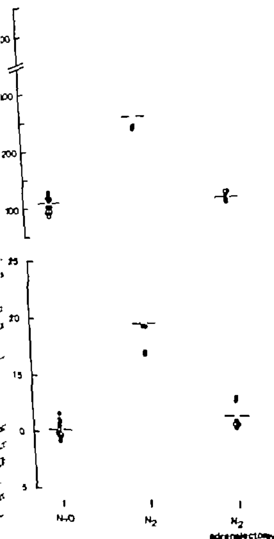


Fig. 1 Individual CBF and CMR_{O_2} values in rats under nitrous oxide anesthesia (N_2O) and in unanesthetized but immobilized animals (N_2) without or with previous adrenalectomy. Mean values are indicated by horizontal bars.

a CBF just above 100 ml (100 g)$^{-1}$ min^{-1} (data from Hägerdal *et al.* 1975). When the nitrous oxide supply was withdrawn CMR_{O_2} almost doubled with a corresponding increase in CBF. This increase in CMR_{O_2} was almost completely prevented by previous adrenalectomy. Furthermore, since it could also be prevented by a beta blocker (propranolol), we conclude that the increase in CMR_{O} represented a stress response mediated by adrenaline.

Although it has been accepted as a central dogma that CMR_{O_2} remains constant in a variety of functional states, Kety (1950) has previously reported an increase in CMR_{O_2} in a patient who showed signs of severe anxiety and King *et al.* (1952) found that adminis-

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Increase in Cerebral Oxygen Uptake and Blood Flow in Immobilization Stress

By

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In the course of a study of cerebral metabolism and circulation in hypoxia we are pre examining the modulating influences of various drugs and anesthetics. Since at least of these drugs (diazepam) appeared to interact with the anesthetic used (nitrous oxi was deemed necessary to study the effect of nitrous oxide by carrying out measurem cerebral blood flow (CBF) and oxygen consumption (CMR_{O_2}) in unanesthetized but lyzed rats. The results of these studies revealed that in the absence of anesthesia, imm zation is accompanied by a pronounced rise in CMR_{O_2} and CBF which appears med by adrenaline. The present article gives a preliminary account of the findings.

Male Wistar rats were anesthetized with 2–3 % halothane, tracheotomized, immob by tubocurarine chloride and maintained ventilated during surgery on 70 % N_2O and O_2. The respirator was adjusted to give a Pa_{CO_2} of 35–40 mm Hg, and body temperatu kept close to 37°C. The femoral arteries were cannulated for blood pressure recording for anaerobic sampling of arterial blood, and the caudal part of the superior sagittal was exposed by a burr hole for sampling of cerebral venous blood CBF was deter with a [133]xenon modification of the Kety and Schmidt (1948) technique, and arteri cerebral venous oxygen content was measured polarographically (Norberg and Siesjö 1 CMR_{O_2} was calculated as the product of the CBF and the arteriovenous difference in o content.

Two groups of unanesthetized animals were studied. In both, operative procedures carried out under anesthesia as described above. In one group, bilateral adrenalectom carried out by a dorsal approach In the other no adrenalectomy was performed. In groups, operative incisions were carefully sutured and the wounds were infiltrated 0.5% Carbocain (Bofors, Sweden) The animals were then removed from the head-h and allowed to rest in a bed of cotton wool Care was taken to exclude or minimize li sound stimuli. After that the animals had been undisturbed for about 10 min, the ni oxide supply was discontinued and the inlet of the respirator was connected to a bag taining [133]xenon in 70% N and 30 % O_2. After a saturation period of 15 min repeated ples were taken from artery and vein for measurements of CBF and CMR_{O_2}. Great car taken during sampling not to disturb the animals.

As seen in the figure, animals under nitrous oxide anesthesia had a CMR_{O_2} of 1

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Reduced Urinary Noradrenaline Excretion during Rest, Exercise and Cold Stress in Trained Rats: a Comparison between Physically Trained Rats, Cold-Acclimated Rats and Warm-Acclimated Rats

By

INGEGERD ÖSTMAN and NILS O. SJÖSTRAND

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Abstract

…MAN, I. and N. O. SJÖSTRAND. *Reduced urinary noradrenaline excretion during rest, exercise and cold stress in trained rats: comparison between physically-trained rats, cold-acclimated rats and warm-acclimated rats.* Acta physiol. scand. 1975. 95. 209–218.

…cally trained rats were compared with cold-acclimated rats. Trained as well as cold-acclimated rats …ed cardiac and adrenal hypertrophy. Cardiac noradrenaline (NA) content was increased in both …ps of rats but only the trained rats had an increased cardiac NA concentration. The adrenal NA …ent … as increased in both groups but only the trained rats had an increased adrenal content of adrena… (A). The spleen of trained rats had an increased NA content, …hile that of cold-acclimated rats had …reased NA content. The submandibular glands of cold-acclimated rats were enlarged and had an in…sed NA content. Trained as …ell as cold-acclimated rats had lower urinary NA excretions during rest, … exercise and during cold stress …hen compared …ith controls. However, only the trained rats had …ced net increment in NA excretion after exercise, …hereas there …as no difference between the incre…ts of cold-acclimated and control rats. Six months after cessation of training, ex-trained rats still had an …eased heart ratio and … reduced urinary NA excretion after exercise. It is suggested that physical training …ces "cross tolerance" to cold stress, …hile cold-acclimation does not lead to "cross tolerance" to acute …rcise.

…n a previous study (Östman, Sjöstrand and Swedin 1972) it was found that, after a period … exercise, trained (chronically exercised) rats display a considerably smaller increase in …inary catecholamine excretion than do untrained rats. Together with the finding of a …er rate of cardiac noradrenaline (NA) turnover in trained rats than in controls both in …st and during exercise, this was taken as an indication of an adaptation of the sympatho…drenal system to chronic exercise, manifested as a better transmitter economy in the trained …imal (Östman *et al.* 1972).

In the sense of reduced demands for catecholamines (CA) in order to maintain certain …nctions, adaptation of the peripheral adrenergic system is well known as a characteristic …f cold acclimation in the rat. Thus, cold-acclimated rats exhibit an increased sensiti…ity

tration of adrenaline to human subjects gave small but significant increases in CBF and CMR_{O_2}. The present results, which are in line with these previous observations, demonstrate that stressful situations may cause a dramatic increase in cerebral metabolism.

We want to acknowledge the excellent technical assistance given by Miss Gunilla Gidö. The work was supported by grants from the Swedish Medical Research Council (Project No. 14 X 263 and ...) and from U.S. PHS No 5 R01 NS 07838-06 from N I H.

References

HÄGERDAL, M., J. HARP, L. NILSSON and B. K. SIESJÖ, The effect of induced hypothermia upon oxygen consumption in the rat brain. *J. Neurochem.* 1975. *24* 311–316.

KETY, S. S. and C. F. SCHMIDT, The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory, procedure and normal values. *J. clin. Invest.* 1948. *27* 476–483.

KETY, S. S., Circulation and metabolism of the human brain in health and disease. *Amer. J. Med.* *8* 205–217.

KING, B. D., L. SOKOLOFF and R. L. WECHSLER, The effects of l-epinephrine and l-nor-epinephrine upon cerebral circulation and metabolism in man. *J. clin. Invest.* 1952. *31* 273–279.

NORBERG, K. and B. K. SIESJÖ, Quantitative measurement of blood flow and oxygen consumption in the rat brain. *Acta physiol. scand.* 1974. *91* 154–164.

...ated, titrated and recentrifuged (see Swedin 1971), whereafter the extracts were oxidized. One tenth ... of 0.27 M EDTA ... as added to the urine samples whereafter the CA were adsorbed on alumina and ... as described by Swedin (1971).

... CA were oxidized and determined fluorometrically according to Chang (1964). The CA values are ...sed as µg free base.

...tical procedures

... values and standard error of the means (S.E.) were computed according to ordinary formulae. The ... were tested and compared by the Student t-test. A p-value of 0.05 or less was considered significant.

Results

...al and organ weights (see Table I and III)

...n our earlier studies (Östman and Sjöstrand 1971, Östman *et al.* 1972) the trained rats ... significantly lower body weight (b.w.) than the controls, while the body length remained ...anged, and significantly greater heart weights, heart ratios and adrenal weights, thus ... rats exhibited the criteria of successful physical training. The cold-acclimated rats also ...ed evidence of cardiac hypertrophy with significantly greater heart weight and heart ...o, and of significantly greater adrenal weight, but their final b.w. did not differ signi...ntly from the controls and neither did their body lengths. However, the b.w. of the cold-...imated rats did not differ significantly from that of the trained rat either. Furthermore, ...eight of the submandibular salivary glands was significantly greater in the cold-accli...ted rats than in controls and trained rats.

...ix months after cessation of the swimming training the only difference that remained ...ween the controls and the ex-trained rats was that the ex-trained rats still had a highly ...nificantly greater heart ratio (Table III).

...BLE I. Effects of physical training and cold-acclimation on body and organ weights in the rat (Mean ... S.E.).

	Initial b.w. (g)	Final b.w. (g)	Final body length (cm)	Heart weight (g)	Heart ratio (g/100 g b.w.)	Salivary glands (g)	Spleen weight (g)	Adrenal weight (mg)
...ntrol rats (12)	180±2	440±7	25.4±0.1	1.23±0.02	0.275±0.003	0.64±0.02	0.78±0.03	40±1
...ained rats (14)	182±2	408±7	25.4±0.2	1.32±0.02	0.325±0.003	0.65±0.01	0.72±0.01	43±1
...old-acclimated rats (n 11)	183_2	427 5	25.1_0.2	1.32±0.02	0.315_0.004	0.72±0.01	0.79±0.02	45±2
...vel of significance in Student t-test								
...ontrols ...rained	n.s.	p 0.01	n.s.	p 0.01	p<0.001	n.s.	n.s.	p 0.02
...ontrol ...old-accl.	n.s.		n.s.	p 0.01	p 0.001	p 0.01	n.s.	p<0.01
...rained ...old-accl.	n.s.	s.	n.s.	n.s.	s.	p 0.01	p 0.01	n.s.

... not significant.

to the calorigenic effect of CA (Hsieh and Carlson 1957 Depocas 1960, Heroux 1961) when exposed to a cold environment they excrete less NA than do warm-acclimated (Leduc 1961) However exposure to cold also involves increased demands on the c... vascular system of the rat The cardiac output increases (Jansky and Hart 1968) and acclimated rats develop cardiac hypertrophy (Emery *et al* 1940).

Thus, there are apparent similarities between the cold-acclimated rat and the trained. Both have a functional hypertrophy of the heart and both display a reduced requirement... the adrenergic transmitter when exposed to an arduous situation to which the organism adapted *i.e* cold and exercise, respectively This has prompted us to make a comparison between cold-acclimated and trained rats. The present report deal with the urinary CA excretion of trained rats, cold-acclimated rats and sedentary warm-acclimated rats subjected to cold stress or to exercise, as well as during rest at room temperature. In add... the NA levels in the heart and the amounts of adrenaline (A) and NA in the adrenals h... been measured. Furthermore, the effect of cessation of training on urinary CA excretion and organ CA levels has been investigated.

Material and Methods

Male Sprague-Dawley rats initially weighing about 180 g and being 40–45 days old were randomly d... in 5 groups. Two groups of rats were submitted to daily exercise during 14 weeks and one group ac... climated to cold during this period. The remaining two groups served as controls. The control animals... the trained rats were kept t a room temperature of about 4 C. The cold acclimated rats on lay... temperature of 4°C. All rats were housed in groups of 5–6 animals and received food and tap water a... All cages were furnished with havi g as bedding. The illumination essentially followed day-light.

The rats were exercised by sw mming in baths, 100 70 60 cm, w th a water depth of 40 cm. 11–1... were swimming simultaneously i the same bath. The water was kept at a temperature of 35+1 C... period of swimming was initially 1 h and was gradually increased to 2 h per day as described pre... (Östman *et al.* 1972).

One group of trained rats, one group of controls and the cold-acclimated rats were killed after 14... the trained rats 4 h after their last period of exercise and the cold acclimated rats after 24 h at room temperature. One group of exercised rats and one control group were in estigated 6 months after the ces... of physical training.

Collection of urine. The determinations of the urinary excretion of CA were made during the 12th... the 13th week of the training period. The urine was collected n metabolic cages, where the animals... kept individually over a period of 22 h. The sampling of resting CA excretion was preceded by ... of rest in the case of the trained rats and by 3 days in room temperature (24°C) in the case of the... acclimated rats. The same periods of rest and exposure to room temperature respectively preceded sa... during cold stress which was performed t +4 C Also when sampling was performed after exerc... animals were first allowed to rest for 2 days o accustomed to room temperature for 3 days but ab... they were submitted to 2 h of swimming before urine collection at room temperature (cf Östman... 1972). After the swimming periods the rats were carefully blotted before the urine collection beg... samplings were made simultaneously on rats f om the different groups. Five ml of 1 M HCl as add... preservative to the collecting bottles. The funnels of the metabolism cages were rinsed with 10 ml of... HCl after removal of the rats. The washing fluid was added to the urine sample.

CA extraction and determination

The rats were killed by exsanguination under light ether anesthesia. The *hearts spleens, submax... glands* and *adrenal glands* were removed, cleaned and weighed W th the exception of the adrenals all ... were homogenized with an Ultra-Turrax apparatus in 15 ml ice-cold 0.4 M perchloric acid cont... 0.1 mM ascorbic acid. The rest of the procedure has previously been described in detail (S... The *adrenal glands* were ground w th chemically pure quartz sand in 2+5 ml ... 0.4 M perchloric

III. Body weight and length, heart and adrenal weights and cardiac noradrenaline content 6 months after cessation of regular exercise (Mean ± S.E.).

	Initial b.w. (g)	Final b.w. (g)	Final body length (cm)	Heart weight (g)	Heart ratio (g/100 g b.w.)	Heart total NA (µg)	Heart NA concentration (µg/g)	Adrenal weight (mg)
Control rats (12)	182 ± 2	567 ± 8	26.9 ± 0.3	1.56 ± 0.03	0.273 ± 0.003	1.17 ± 0.12	0.76 ± 0.08	54 ± 2
Trained rats (12)	179 ± 2	548 ± 13	27.1 ± 0.1	1.64 ± 0.03	0.299 ± 0.003	1.47 ± 0.11	0.90 ± 0.07	54 ± 1
of significance in Student's t-test								
control – trained rats	n.s.	n.s.	n.s.	n.s.	p 0.001	n.s.	n.s.	n.s.

-acclimated rats, the cold-acclimated animals in their turn also excreted significantly NA than the controls did. A conspicuous difference between trained and cold-acclimated rats was revealed when paired observations from the same *individual* animals were compared, it was found that the *net increase* in the amount of NA excreted after exercise (after subtracting the amount of NA excreted during a rest period) was of the same magnitude in cold-acclimated rats and in controls, but significantly smaller in the trained rats (controls, 0.60 ± 0.07 µg NA/22 h, n = 9; cold-acclimated, 0.64 ± 0.06 µg NA/22 h, n = 8; trained, 0.35 ± 0.05 µg NA/22 h, n = 11, p 0.05).

During *cold stress* the amounts of both NA and A excreted were markedly increased in all experimental groups as compared with the resting state. The amounts of A excreted

Table IV The influence of regular physical training and of cold-acclimation on urinary excretion of catecholamines after exercise and during cold stress (Mean ± S.E.).

	During rest		After exercise		During cold stress	
	NA-excretion (µg/22 h)	A-excretion (µg/22 h)	NA-excretion (µg/22 h)	A-excretion (µg/22 h)	NA excretion (µg/22 h)	A excretion (µg/22 h)
Control rats	0.95 0.05 (15)	0.14 ± 0.01 (15)	1.37 ± 0.06[d] (12)	0.19 ± 0.02[a] (12)	2.89 ± 0.15[d] (12)	0.20 ± 0.01[d] (12)
Trained rats	0.64 ± 0.03 (12)	0.12 ± 0.01 (12)	0.88 ± 0.07[c] (18)	0.14 ± 0.02 (18)	2.32 ± 0.08[d] (12)	0.23 ± 0.02[d] (12)
Cold-acclimated rats	0.68 ± 0.06 (11)	0.07 ± 0.01 (11)	1.17 ± 0.05[d] (11)	0.16 ± 0.01[d] (11)	2.41 ± 0.17[d] (11)	0.21 ± 0.03[d] (11)
Level of significance in Student's t-test						
control – trained	p 0.001	n.s.	p 0.001	n.s.	p < 0.01	n.s.
controls – cold accl.	p 0.001	p 0.001	p 0.02	n.s.	p 0.05	n.s.
trained – cold accl.	n.s.	p 0.01	p 0.01	n.s.	n.s.	n.s.

number of observations.
difference from resting value p 0.05.
difference from resting value p 0.01.
difference from resting value p 0.001
n.s. not significant

TABLE II Effect of physical training and cold-acclimation on catecholamines in different organs [illegible] (± S.E.).

	Heart total NA (μg)	Heart NA concentration (μg/g)	Salivary glands total NA (μg)	Salivary glands NA concentration (μg/g)	Spleen total NA (μg)	Adrenals total A (μg)	Adrenals total NA (μg)	[illegible]
Control rats (n = 12)	0.87 ± 0.03	0.71 ± 0.04	0.68 ± 0.03	1.02 ± 0.04	0.66 ± 0.03	45.1 ± 1.3	7.4 ± 0.4	[illegible]
Trained rats (n = 12)	1.11 ± 0.06	0.84 ± 0.05	0.75 ± 0.03	1.12 ± 0.05	0.83 ± 0.03	51.0 ± 1.5	9.8 ± 0.5	[illegible]
Cold acclimated rats (n = 11)	1.01 ± 0.05	0.77 ± 0.02	0.81 ± 0.04	1.13 ± 0.07	0.57 ± 0.03	49.6 ± 1.8	9.8 ± 0.8	[illegible]
Level of significance in Student's t test								
Controls v trained	p < 0.01	p < 0.05	n.s.	n.s.	p < 0.01	p < 0.01	p < 0.01	[illegible]
Controls cold accl.	p < 0.05	n.s.	p < 0.05	n.s.	p < 0.05	n.s.	p < 0.05	[illegible]
Trained cold accl.	n.s.	n.s.	n.s.	n.s.	p < 0.001	n.s.	n.s.	n.s.

n.s. = not significant

Catecholamine content of the organs (see Table II and III)

The results confirm our earlier observations (Östman *et al* 1972) that training by [illegible] causes a significant increase in both the total amount and the concentration of NA [illegible] heart and in the amounts of A and NA in the adrenal glands. However in the [illegible] glands the increase in NA-content is relatively greater than that of A as shown by the [illegible] that the relative proportion of NA is significantly increased. A significantly higher [illegible] content was also found in the spleens of the trained rats. Cold-acclimation led to a significant increase in cardiac NA-content, but in contrast to trained rats, the increase in cardiac [illegible] concentration was not significant. Increased NA-content but not NA-concentration [illegible] also found in the submandibular glands of the cold-acclimated animals, but the NA-co[illegible] of the spleens, on the other hand was significantly decreased. In the adrenals of cold-[illegible]mated rats only the NA-content was significantly increased and as with the trained [illegible] the proportion of the total CA in the adrenal glands made up by NA was significantly [illegible]creased. Six months after cessation of physical training no significant difference [illegible] between the cardiac NA-contents of the ex-trained rats and those of the controls (Table [illegible]

Urinary CA-excretion (see Table IV and V)

During *resting conditions* both the trained and the cold-acclimated rats excreted significantly less NA than the controls did. Furthermore, the cold-acclimated rats also excreted significantly less A than did both the controls and the trained rats.

In the urine collected *after exercise* all groups showed a significantly increased N[illegible] excretion as compared with the resting state, but only the controls and the cold-acclimated rats showed a significant increase in A-excretion. The *total* amount of NA excreted [illegible] exercise by the trained rats, however was significantly lower than that of both control [illegible]

...cholamine content of tissues

...previous finding of increased cardiac NA-content as well as NA-concentration in ...ically exercised rats (Östman *et al.* 1972) is confirmed in this study and we also find an ...ased content of NA in the spleen, another organ involved in adjustments to conditions ...culatory stress. The increase in NA is probably related to more effective mechanisms ...is and storage of the transmitter induced by the daily exercise as discussed by Öst... *et al.* (1972). Thus, an increased impulse activity in the sympatho-adrenal system induces ...ased activity in ganglia and organs of tyrosine hydroxylase (*cf.* Thoenen *et al.* 1973) as ...as of dopamine β-hydroxylase (Molinoff *et al.* 1970) which is a component of the ...nergic storage vesicle (Smith 1972) and therefore also may reflect an increased storage ...city for NA.

...e Schryver and coworkers (*cf.* De Schryver and Mertens-Strythagen 1972) have reported ...creased cardiac NA-content in "trained" rats and suggested that this could in part ...ain the bradycardia of the athletic heart. However these authors use a very low training ...sity (running for 90 min at a speed of 8.3 or 12.5 m/min) and they exercise their rats ...three times a week. Such training parameters are not sufficient to induce cardiac en...rement (Petow and Siebert 1925, Leon and Bloor 1968). Accordingly De Schryver and associates never see any sign of cardiac hypertrophy in their "trained" rats. We find their ...lts on cardiac NA-content puzzling but since the athletic heart by definition is an en...ged heart and since De Schryver and coworkers never measured the heart rate in their ...s, we reject their conclusion that their finding of reduced transmitter levels should in part ...plain the bradycardia of the athletic heart (see also Östman and Sjöstrand 1971).

The cardiac hypertrophy of the cold-acclimated rats is presumably related to the increased ...diac output occurring after cold-exposure (Jansky and Hart 1968, Popovic *et al.* 1969), ...d the increased cardiac NA due to an increased activity in the sympathetic nerves as ...cussed above. Thus, cold exposure increases cardiac NA-turnover just as does exercise ...ordon *et al.* 1966).

The decreased NA-content in the spleen of cold-acclimated rats might be due to a de...eased sympathetic activity in the organ caused by the increased *peripheral vasoconstriction* ...mbined with high circulating levels of CA. Thus, the data of Jansky and Hart (1968) indi...te that the spleen, in contrast to most other vascular beds, is not actively involved in the ...rculatory *adjustments following* acute or chronic exposure to cold.

The increased adrenal CA levels and the increase in relative proportion of NA found in ...ained and cold-acclimated rats are in agreement with earlier findings (Östman *et al.* 1972, ...vetnansky *et al.* 1971). Also the increase in adrenal CA-content is likely to be mediated ...rough induction of enzymes (*cf.* Molinoff *et al.* 1970, Kvetnansky *et al.* 1971).

...rinary excretion of catecholamines

...ained rats. The previous observation of smaller increase in urinary NA excretion after ...xercise in trained rats than in controls is confirmed in this study. This time we also find a ...ower NA-excretion in trained rats during rest (*i.e.* at least 48 h after the last exercise). Our ...results suggest that chronically exercised animals require a lower degree of sympathetic ...tone in order to maintain homeostasis in rest as well as during exercise. This fits with the

TABLE V Urinary excretion of catecholamines 6 months after cessation of regular exercise [illegible]

	During rest		After exercise		During cold [illegible]	
	NA excretion (μg/22 h)	A excretion (μg/22 h)	NA excretion (μg/22 h)	A excretion (μg/22 h)	NA-excretion (μg/22 h)	A-excr[illegible]
Old control rats	1.12 ± 0.05 n = 14	0.14 ± 0.01 n = 14	1.73 ± 0.09[d] n = 14	0.[illegible] ± 0.03[b] n = 14	3.74 ± 0.12[d] n = 14	[illegible]
Ex trained rats	1.03 ± 0.06 n = 14	0.11 ± 0.01 n = 14	1.46 ± 0.07[d] n = 14	0.22 ± 0.02[d] n = 14	3.39 ± 0.16[d] n = 14	[illegible]
Level of significance in Student's t-test						
Old control rats -ex trained	n.s.	n.s.	$p < 0.05$	n.s.	n.s.	n.s.

n = number of observations.
[b] = different from resting value $p < 0.0$[illegible]
[d] = different from resting value $p < 0.001$
n.s. = not significant

were of the same order of magnitude in all three groups the amounts of NA excreted [illegible] both the cold-acclimated rats and the trained rats were, however significantly lower [illegible] the amounts excreted by the controls.

Six months after cessation of swimming training the only significant difference remained between control and ex trained rats was that the ex trained rats excreted less NA after exercise than did the controls (Table V). This was also found when the net increase in the amount of NA excreted by individual rats after exercise was determined in paired observations (controls 0.75 ± 0.10 μg NA/22 h, n = 10 ex-trained 0.41 ± 0.07 μg NA/22 h, n = 11 p < [illegible]

Discussion

Organ weights

The increase in heart and adrenal weights agree with earlier findings in trained (Hatai [illegible] and cold acclimated (Emery *et al* 1940, Heroux and Hart 1954) rats. However in [illegible] respects the present data diverge from earlier findings. Firstly we do not see the [illegible] inhibition of growth of cold-acclimated animals reported by *e.g.* Schwabe *et al.* (193[illegible] Heroux and Hart (1954) This might be due to the fact that in our experiment the se[illegible] of the cold stress is diminished by keeping the rats in groups and in furnished cages.

Secondly our data indicate that a certain degree of cardiac hypertrophy persists as l[illegible] as 6 months after cessation of training. Other investigators *e.g.* Secher (1923), Hort (19[illegible] Leon and Bloor (1968) and Ljungquist and Unge (197[illegible]), have reported a rapid return [illegible] heart weight to control values when chronic exercise is discontinued. The discrepancy [illegible] probably explained by the fact that we have trained male rats rather intensely during pub[illegible] while other authors have used old rats (Hort 1951 Ljungquist and Unge 1972) or exercise[illegible] the rats less severely (Leon and Bloor 1968).

The increase in weight of the salivary gland of the cold-acclimated rats is probably related to an increased food intake (*cf* Schwabe *et al.* 1938) and might be caused by an increase[illegible] activity in the sympathetic nerves to the gland (*cf* Himms-Hagen 1972

hat chronically exercised rats have a lower demand for NA both in the warm and in ld, and that they have greater tissue reserves of CA together with a greater capacity thesis of CA, we suggest that increased levels of physical activity in mammals may a better fitness to withstand cold and, possibly other stressful conditions.

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References

, C. C., A sensitive method for spectrophotofluorometric assay of catecholamines. *Int. J. Neuropharmacol.* 1964, 3. 643–649.

, F. The calorigenic response of cold-acclimated white rats to infused noradrenaline. *Canad. J. Biochem. Physiol.* 1960. 38. 107–114.

ERTEN, C. and J. MERTENS-STRYTHAGEN, Intensity of exercise and heart tissue catecholamine content. *Pflügers Arch. ges. Physiol.* 1972. 336. 345–354.

F. E., L. M. EMERY and E. L. SCHWABE, The effects of prolonged exposure to low temperature on body growth and on the weights of organs in the albino rat. *Growth* 1940. 4. 17–32.

SON, R., S. SPECTOR, A. SJOERDSMA and S. UDENFRIEND, Increased synthesis of norepinephrine and epinephrine in the intact rat during exercise and exposure to cold. *J. Pharmacol. exp. Ther.* 1966. 153. 3–447.

, M. N. E., L. MELENDER and R. TIRRI, Changed chronotropic sensitivity to sympathomimetic amines in isolated atria from rats following cold acclimation. *Experientia* (Basel) 1974. 30. 1041–1043.

, S., On the influence of exercise on the growth of organs in the albino rat. *Anat. Rec.* 1935. 9. 647–665.

, O., Comparison between seasonal and thermal acclimation in white rats. *Canad. J. Biochem. Physiol.* 1961. 39. 1829–1836.

, O. and J. S. HART, Comparison of four indices of adrenal activity in rats acclimated to 30° 1°C. *Amer. J. Physiol.* 1954. 178. 445–448.

-HAGEN, L., Effects of catecholamines on metabolism. VIII Salivary glands. In: *Handbook of Experimental Pharmacology* XXXIII Catecholamines. Eds. H. Blaschko and E. Muscholl, pp. 423–427 Springer Verlag, Berlin, Heidelberg, New York. 1972.

-HAGEN, L., Role of the adrenal medulla in adaptation to cold. In: *Handbook of Physiology* Section Vol. 6, 1975.

-HAGEN, J. and I. M. MAZURKIEWICZ-KWILECKI, Unchanged sensitivity to noradrenaline in isolated from cold-acclimated rats. *Canad. J. Physiol. Pharmacol.* 1970. 48. 657–660.

, W. Morphologische und physiologische Untersuchungen an Ratten. Abend eines Lauftrainings und nach dem Training. *Virchows Archiv* 1951. 320. 197–237.

, A. C. L. and L. D. CARLSON, Role of adrenaline and noradrenaline in chemical regulation of heat production. *Amer. J. Physiol.* 1957. 190. 243–246.

, A. C. L., C. W. PUN, K. M. LI and K. W. TI, Circulatory and metabolic effects of noradrenaline cold-adapted rats. *Fed. Proc.* 1966. 25. 1205–1212.

, L., R. BARTUNKOV and E. ZEMMERMAN, Acclimation of the white rat to cold, noradrenaline thermogenesis. *Ph. siol. Bohemoslov.* 1967. 16. 366–372.

, L. and J. S. HART, Cardiac output and organ blood flow in warm- and cold-acclimated rats exposed to cold. *Canad. J. Physiol. Pharmacol.* 1968. 46. 653–659.

TANSKI, R. G. P. GEWIRTZ, V. K. WEISE and I. J. KOPIN, Catecholamine-synthesizing enzymes in the rat adrenal gland during exposure to cold. *Amer. J. Physiol.* 1971. 220. 928–931.

BLANC, J. Effect of adrenaline, noradrenaline and chlorpromazine on blood pressure of normal and cold adapted rat. *Proc. Soc. exp. Biol.* (N.Y.) 1960. 105. 109–111.

BLANC, J. and M. POULIOT, Importance of noradrenaline in cold adaptation. *Amer. J. Physiol.* 1964. 207. 853–856.

BLANC, J., J. VALLIERES and C. VACHON, Beta-receptor sensitization by repeated injections of isoproterenol and by cold adaptation. *Amer. J. Physiol.* 1972. 222. 1043–1046.

decreased NA turnover found in the heart (Östman *et al.* 1972) and the spleen (Öst- Nybäck 1975) of trained rats and also with the increased vascular sensitivity to NA in these rats (Östman 1975)

The reduced NA excretion during cold stress of the trained rats might in part be expl- by a lower need for the transmitter in the cardiovascular adjustments that occur during exposure. However it is also quite possible that chronic exercise increases the sensi- tissues to the metabolic action of NA This could be due to the daily exposure of the to higher than normal concentrations of CA during the exercise periods. Thus, daily t- ment of rats with NA sensitizes their tissues to the metabolic actions of NA (LeBlanc Poullot 1964)

Six months after cessation of exercise the trained rats still excreted less NA than co- did. Possibly this reflects the persistence of reduced sympathetic tone to the hypertr- heart and, perhaps, also a better circulatory adjustment, rather than a persistent inc in vascular and/or metabolic sensitivity to the transmitter Thus, the increased sensi- tivity to the metabolic actions of NA of cold-acclimated rats declines rather rapidly whe rats are retransferred to warm environment (Jansky *et al* 1967).

Cold-acclimated rats The depressed levels of urinary CA in these animals when they at room temperature, as well as their decreased NA excretion during coldstress, are prob to a great extent related to the increased sensitivity to the calorigenic effects of NA which occurs in cold-acclimation (*cf* Himms-Hagen 1975) However the cold-accl rats had the same net increment in NA excretion after exercise as the controls had. It appears that cold-acclimated rats do not develop any "cross-tolerance to the circ (and metabolic) stress of exercise, although chronically exercised rats do seem to dev degree of cross-tolerance" to the metabolic (and circulatory) stress of cold exposure reasons for this difference are not known. Both groups develop cardiac hypertroph conflicting results have been reported concerning cardiovascular sensitivity to NA in acclimated rats. Increased sensitivity or increased maximum response to CA of hea vessels have been claimed by some investigators (LeBlanc 1960, Heroux 1961 LeBlanc 1972, Harri *et al* 1974) while other authors have denied such effects (Hsieh *et al.* Himms-Hagen and Mazurkiewicz Kwilecki 1970) Our results are more consistent wi latter view but other explanations can not be excluded.

Conclusions

The adaptive changes following physical training include a greater economy of the sym thetic transmitter which may well be to a large extent due to increased sensitivity of the to the vascular and metabolic actions of NA. The consequences of this are obvious animal develops a better tolerance to conditions that require an increased transmi secretion in order to maintain homeostasis. Exposure to severe cold is, for a small n hibernating mammal, a critical situation and it is the capacity for non-shivering therm genesis induced by the calorigenic actions of CA that determines the survival of such animal. In natural conditions the activity period is increased during the autumn and winter for a nocturnal species such as the rat. Since our results in this and previous stu

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Effects of Drugs and Nerve Stimulation on the Spleen and Arteries of Two Species of Dogfish, Scyliorhinus canicula and Squalus acanthias

By

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Abstract

...on, S., S. Holmgren and D. J. Grove. *Effects of drugs and nerve stimulation on the spleen and arteries of two species of dogfish,* Scyliorhinus canicula and Squalus ...acanthias. Acta physiol. scand. 1975. 95. 219–230.

...ffects of drugs and nerve stimulation on the spleen of 2 species of dogfish have been examined by ...ments with perfused spleens and isolated spleen and artery strips. Adrenaline, noradrenaline and ...lephrine, acting on alpha adrenoceptors, constrict the perfused spleen of both species, thereby re... erythrocytes, and contract the isolated spleen and artery strips. Phentolamine competitively an... the excitatory effects of adrenergic ...gonists. The responses of the spleen to acetylcholine are very ... and rapid desensitization makes evaluation of the mode of action of this drug difficult. In the ... strips acetylcholine produces dose-dependent contraction. Fluorescent histochemistry reveals ...loped adrenergic innervation of the arteries, and few adrenergic terminals in the spleen. Stimulation ... nerves produces normally splenoconstriction in *Squalus*, which can be blocked by phentolamine ... by atropine. The nervous control of the *Scyliorhinus* spleen seems to be poor or lacking. It is con...ed that the dogfish spleen, and maybe also the arteries, are to a large extent controlled by circulating ... catecholamines and (in *Squalus*) also by sympathetic adrenergic fibres.

...words: Dogfish, spleen innervation, autonomic nervous system, adrenergic drugs, cholinergic drugs

...ere are few reports concerning the effects of drugs on and autonomic control of the spleen ...fish. Acetylcholine and alpha adrenoceptor stimulating agents contract isolated spleen ...ips from the tench (*Tinca tinca*) (Vairel 1933) and the cod (*Gadus morhua*) (Holmgren and ...ilsson 1975). Bonnet (1929) concluded from her experiments with the tench that the spleen ...fish releases erythrocytes in response to asphyxia, and Nilsson and Grove (1974) showed ...at in the perfused spleen of cod, splenic constriction and release of erythrocytes can be ...roduced both by nerve stimulation and by injection of acetylcholine or alpha adrenoceptor ...gonists. The nerve supply to the cod spleen consists of both adrenergic and cholinergic ...ostganglionic sympathetic constrictory fibres (Nilsson and Grove 1974). Opdyke and ...pdyke (1971) concluded by examining the haematocrit of the splenic effluent in the spiny

LEDUC, J Catecholamine production and release in exposure and acclimation to cold. *Acta physiol* [illegible] 1961 *53* Suppl. 183

LEON A S. and C. M BLOOR, Effects of exercise and its cessation on the heart and its blood [illegible] *appl Physiol.* 1968. *24* 485–490.

LJUNGQUIST A. and G UNGE, The finer intramyocardial vasculature in various forms of exp [illegible] cardiac hypertrophy *Acta path. microbiol. scand.* 1972. Section A *80* 329–340.

MOLINOFF P B., S. BRIMIJOIN, R. WEINSHILBOUM and J AXELROD, Neurally mediated increase [illegible] mine-β-hydroxylase activity *Proc Nat Acad. Sci.* 1970. *66.* 453–458.

ÖSTMAN, I., Changed vascular sensitivity to noradrenaline in rats trained by physical exercise *J* [illegible] (Lond.) 1975 In press.

ÖSTMAN I. and H. NYBÄCK, Adaptive changes in central and peripheral noradrenergic neurons [illegible] following chronic exercise. *Neuroscience 1* 1975. In press.

ÖSTMAN, I and N O SJÖSTRAND Effect of prolonged physical training on the catecholamine [illegible] heart and the adrenals of the rat. *Acta physiol. scand.* 1971 *82.* 20[illegible] 208.

ÖSTMAN, I., N O SJÖSTRAND and G SWEDIN, Cardiac noradrenaline turnover and urinary cat[illegible] excretion in trained and untrained rats during rest and exercise. *Acta physiol. scand.* 1972 [illegible]

PETROW H. and W SIEBERT Studien über Arbeitshypertrophie des Muskels. *Z Klin. Med* [illegible] 427–433

POPOVIC, V K. KENT N MOJOVIC, B. MOJOVIC and J S. HART Effect of exercise and cold on [illegible] output in warm- and cold-acclimated rats. *Fed. Proc* 1969 *28.* 1138–1141

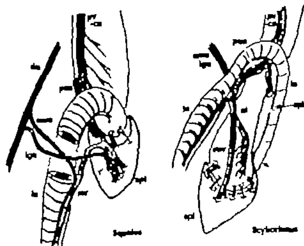
SCHWABE, E. L., F E. EMERY and F R GRIFFITH Jr The effect of prolonged exposure to low temp [illegible] on the basal metabolism of the rat. *J Nutr* 1938. *15* 199–210.

SECHER, K. Experimentelle Untersuchungen über die Grösse des Herzens nach einem Aufenthalt [illegible] nierens. *Z. exp. Med* 1923. *32.* 290–295.

SMITH, A. D., Subcellular localisation of noradrenaline in sympathetic neurons. *Pharmacol. Rev.* [illegible] *24* 435–457

SWEDIN, G Studies on neurotransmission mechanisms in the rat and guinea-pig vas deferens. *Acta physiol. scand.* 1971 *83* Suppl. 369

THOENEN H., U OTTEN and P OESCH, Trans-synaptic regulation of tyrosine hydroxylase. In: *Frontiers in Catecholamine Research.* Eds. E. Usdin and S. Snyder pp. 179–185. Pergamon Press, Oxford [illegible]



Simplified diagram of the ...y of the blood vessels ... the spleen in *Squalus* ... and *Scyliorhinus* ... ...sp. anterior mesenteric artery ca, coeliac artery ... ...al aorta, ta, spiral intestine, intestinal vein; lga, ...ic artery pan, pan... pv, portal vein, sev splenic ... and vein, spl, spleen, st, ...ch.

...following drugs were used: acetylcholine chloride, L-adrenaline bitartrate, atropine sulphate, car... chloride, 5-hydroxytryptamine creatinine sulphate, DL-isoprenaline hydrochloride, mecamyla... hydrochloride, nicotine sulphate, L-noradrenaline bitartrate, phentolamine methane sulphonate, ...ylephrine hydrochloride, propranolol hydrochloride, sulfonamol chloride. The amounts or con...tions of these drugs are expressed in moles or mol/l (M) respectively.

...escent histochemistry

...of the lieno-gastric artery, the coeliac artery, the spleen and sympathetic ganglia from *Squalus* were ...frozen in liquid propane cooled in liquid nitrogen and freeze-dried according to the method described ...ick and Owman (1965). One animal was injected with noradrenaline (5 mg/kg) 1 h prior to killing and ...g the organs. After treatment with formaldehyde vapour at 80°C for 1 or 3 h, the preparations were ...dded in paraffin ...ax. Controls were embedded without previous formaldehyde treatment. Sections ...) were mounted in Entellan (Merck) and viewed in ... Leitz Orthoplan microscope with top illumination ...barrier filter at 460 nm. Photographs were taken by ... Leitz Orthomat automatic camera on Kodak ...film.

Results

...eriments with isolated strip preparations

...energic drugs. Adrenaline, noradrenaline and phenylephrine contracted the spleen strips ...m both *Scyliorhinus* and *Squalus* as well as the artery strips from *Squalus* (Table I, Fig. 6). ...*liorhinus* artery strips were also contracted by adrenaline and noradrenaline (Table I), ...very few pD_2-values have been calculated since in most preparations the first low dose of ...agonist induced rhythmic activity which could not be abolished, and no dose-response ...ves could be obtained on these preparations.

...soprenaline (10– 10⁻⁴ M) had no detectable effect on the spleen strips from either ...cies. Concentrations between 10^{-7} M and 10^{-6} M slightly relaxed artery strips from ...*alus*, ...hile higher doses caused a small contraction.

The alpha adrenoceptor antagonist phentolamine produced a parallel shift of the dose ...ponse curve for noradrenaline in the *Scyliorhinus* spleen strips and the *Squalus* spleen and ...tery strips. The pA-values are summarized in Table II.

dogfish (*Squalus acanthias*) that this species has not developed the ability to release [illegible] cytes from the spleen in response to sympathetic nerve stimulation or adrenaline.

The present work examines the mode of autonomic innervation and the effects of [illegible] gic and cholinergic drugs on the spleen of dogfish by experiments with isolated [illegible] artery strips and perfused spleens.

Materials and Methods

Dogfish, *Scyliorhinus canicula* (650–750 g, length 60–65 cm) and spiny dogfish, *Squalus acanthias* [illegible] 1 400 g, length 50–75 cm) of either sex were used in the experiments. The animals were kept in [illegible] well-aerated sea water at 10–15°C for less than 4 weeks before the experiments.

Experiments with isolated strip preparations

Spleen strips, approx. 25 mm long and 2–3 mm wide including parts of the capsule, or longitudinal [illegible] of the lieno-gastric or coeliac artery (Fig. 1) were suspended in organ baths and attached to GRASS isometric transducers connected to a GRASS Polygraph model 7. The organ baths contained a saline [illegible] following composition (*Scyliorhinus*): NaCl 15.05, $NaHCO_3$ 2.20, Na_2HPO_4 0.23, KH_2PO_4 [illegible], $CaCl_2 \cdot 6H_2O$ 1.10, $MgSO_4 \cdot 7H_2O$ 1.00, glucose 1.0 and urea 24.0 g/litre or (*Squalus*): NaCl 13.5, [illegible] $CaCl_2 \cdot 2H_2O$ 0.70, $MgSO_4 \cdot 7H_2O$ 0.60, $NaHCO_3$ 1.00, $Na_2HPO_4 \cdot 2H_2O$ 0.[illegible], glucose 1.0 and urea [illegible] (calculated from *Squalus* plasma levels from Holmes and Donaldson 1969). The saline was kept at [illegible] temperature within the range 10–15°C and bubbled with a mixture of O_2 and CO_2 (*Scyliorhinus* 99.5:0.5, *Squalus* 97:3 %), giving a pH of ca 7.5 in both cases). The tonus of the spleen strips was adjusted to 50 mg, and the arteries were adjusted to their *in vivo* length. The preparations were left to reach [illegible] base-line for 1 h (*Scyliorhinus*) or up to 18 h (*Squalus*) before any addition of drugs.

Cumulative dose-response curves were obtained and pD_2-values ($pD_2 = -\log ED_{50}$ [illegible]) [illegible] determined for the various agonists. Experiments, where subsequent cumulative dose-response curves [illegible] one agonist were produced, showed that the pD_2-values did not differ detectably from one curve to [illegible] other but that the maximal contraction force obtained was very irregular. Consequently it has been [illegible] possible to evaluate the intrinsic activity (alpha-value, van Rossum 1963) for the different agonists [illegible] and their effects on the preparations will be presented only as their pD_2-values.

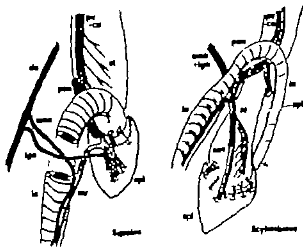
In the experiments with the alpha adrenoceptor antagonist phentolamine, this was added to the [illegible] 10 min before obtaining the second (or third) dose-response curve. The pA values were determined according to Benfey and Grillo (1963) as described by Holmgren and Nilsson (1975).

Experiments with perfused spleen

The animals were injected with 1 000–2 000 I.U. of heparine in the caudal vessels a few min before [illegible] by a sharp blow on the head and subsequent pithing. The spleen was exposed and the splenic artery and [illegible] (*Scyliorhinus*) or lieno-gastric artery and intestinal vein (*Squalus*) (Fig. 1) catheterized for the inflow [illegible] outflow respectively of perfusion fluid. All vessels entering and leaving the spleen save the catheterized vessels were carefully ligated. The spleen was separated from the stomach, removed from the animal [illegible] allowed to rest on tissues moistened with saline. The *Scyliorhinus* spleen was ligated at the connection between the two parts of the spleen (Fig. 1 [illegible] arrow) to allow perfusion of the posterior (main) part [illegible]

The perfusion fluid was the saline described above, and in the case of *Scyliorhinus*, 15 g polyvinyl-[illegible] lidone per litre was added to avoid edema (Davies and Rankin 1973) but this was found unnecessary [illegible] *Squalus*. Perfusion was carried out at constant pressure (variation during each experiment less than 0.5 [illegible] H_2O) within the range 25–30 cm H_2O with the apparatus of Davies and Rankin (1973) as modified [illegible] Nilsson and Grove (1974). The outflow pressure was close to zero cm H_2O. Perfusion flow was determined as drop rate either on the inflow and outflow side by GRASS PT1 photoelectric transducers connected to [illegible] GRASS 7P4 tachograph, or on the outflow side only by a drop-counter similar to that described by Davies and Rankin (1973). Drugs were added either as single doses by a syringe and a catheter into the perfusion stream or dissolved in the perfusion fluid. Responses were determined as the flow decrease in per cent of the initial flow rate measured on the outflow side.

Stimulation of the splenic nerves were made by two silver electrodes over which the whole splenic [illegible] was placed. Trains of pulses lasting up to 120 s with 10–50 Hz, 0.5–1.0 ms duration and 3–18 V were applied



Simplified diagram of the ...y of the blood vessels ... the spleen of Squalus ... and Scyliorhinus ... anterior mesenteric artery; ca, coeliac artery; ... dorsal aorta; ... spiral intestine; ... intestinal vein; lgn, ... artery; pam, ... portal vein; mv, splenic ... and vein; spl, spleen; st, ... ls.

The following drugs were used: acetylcholine chloride, L-adrenaline bitartrate, atropine sulphate, car-...ame chloride, 5-hydroxytryptamine creatinine sulphate, DL-isoprenaline hydrochloride, mecamylamine hydrochloride, nicotine sulphate, L-noradrenaline bitartrate, phentolamine methane sulphonate, ...ylephrine hydrochloride, propranolol hydrochloride, salbutamol chloride. The amounts or concentrations of these drugs are expressed as moles or mol/l (M) respectively.

...cence histochemistry

... of the hepato-gastric artery, the coeliac artery, the spleen and sympathetic ganglia from Squalus were ... frozen in liquid propane cooled in liquid nitrogen and freeze-dried according to the method described ... ck and Owman (1965). One animal was injected with noradrenaline (5 mg/kg) 1 h prior to killing and ... ing the organs. After treatment with formaldehyde vapour at 80°C for 1 or 3 h, the preparations were ... dded in paraffin wax. Controls were embedded without previous formaldehyde treatment. Sections ... μ) were mounted in Entellan (Merck) and viewed in a Leitz Orthoplan microscope with top illumination ... barrier filter at 440 nm. Photographs were taken by a Leitz Orthomat automatic camera on Kodak film.

Results

...eriments with isolated strip preparations

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dogfish (*Squalus acanthias*), that this species has not developed the ability to release cytes from the spleen in response to sympathetic nerve stimulation or adrenaline.

The present work examines the mode of autonomic innervation and the effects of adrenergic and cholinergic drugs on the spleen of dogfish by experiments with isolated splenic artery strips and perfused spleens.

Materials and Methods

Dogfish, *Scyliorhinus canicula* (650–750 g, length 60–65 cm) and spiny dogfish, *Squalus acanthias* 1 400 g, length 50–75 cm) of either sex were used in the experiments. The animals were kept in well-aerated sea water at 10–15°C for less than 4 weeks before the experiments.

Experiments with isolated strip preparations

Spleen strips, approx. 25 mm long and 2–3 mm wide including parts of the capsule, or longitudinal of the lieno-gastric or coeliac artery (Fig. 1) were suspended in organ baths and attached to GRASS isometric transducers connected to a GRASS Polygraph model 7. The organ baths contained saline following composition (*Scyliorhinus*): NaCl 15.05, $NaHCO_3$ 2.20, Na_2HPO_4 0.23, KH_2PO_4 0.05, $CaCl_2 \cdot 6H_2O$ 1.10, $MgSO_4 \cdot 7H_2O$ 1.00, glucose 1.0 and urea 24.0 g/litre or (*Squalus*): NaCl 1... $CaCl_2 \cdot 2H_2O$ 0.70, $MgSO_4 \cdot 7H_2O$ 0.60, $NaHCO_3$ 1.00, $Na_2HPO_4 \cdot 2H_2O$ 0.2..., glucose 1.0 and urea ... (calculated from *Squalus* plasma levels from Holmes and Donaldson 1969). The saline was kept at a temperature within the range 10–15°C and bubbled with a mixture of O_2 and CO_2 (*Scyliorhinus* ... *Squalus* 97:3 %) giving a pH of c.a 7.5 in both cases). The tonus of the spleen strips was adjusted at 50 mg, and the arteries were adjusted to their *in situ* length. The preparations were left to reach a base-line for 1 h (*Scyliorhinus*) or up to 18 h (*Squalus*) before any addition of drugs.

Cumulative dose-response curves were obtained and pD_2-values (pD_2 = –log ED_{50}) as ... determined for the various agonists. Experiments, where subsequent cumulative dose-response curves of one agonist were produced, showed that the pD_2-values did not differ detectably from one curve to the other, but that the maximal contraction force obtained was very irregular. Consequently it has been possible to evaluate the intrinsic activity (alpha-value, van Rossum 1963) for the different agonists and their effects on the preparations will be presented only as their pD_2-values.

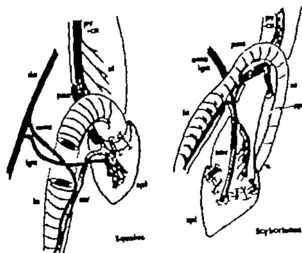
In the experiments with the alpha adrenoceptor antagonist phentolamine, this was added to the 10 min before obtaining the second (or third) dose-response curve. The pA_2-values were determined according to Besley and Grillo (1963) as described by Holmgren and Nilsson (1975).

Experiments with perfused spleen

The animals were injected with 1 000–2 000 I.U. of heparine in the caudal vessels a few min before by a sharp blow on the head and subsequent pithing. The spleen was exposed and the splenic artery (*Scyliorhinus*) or lieno-gastric artery and intestinal vein (*Squalus*) (Fig. 1) catheterized for the inflow and outflow respectively of perfusion fluid. All vessels entering and leaving the spleen save the catheterized vessels were carefully ligated. The spleen was separated from the stomach, removed from the animal, allowed to rest on tissues moistened with saline. The *Scyliorhinus* spleen was ligated at the constriction between the two parts of the spleen (Fig. 1, ... arrow) to allow perfusion of the posterior (main) part.

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…implified diagram of the … of the blood vessels … the spleen in *Squalus* and *Scyliorhinus* can- …gastr… ana, anterior me- … artery ca, coeliac artery … al aorta, m, spiral intes- …n, intestinal vein, lga, …tric artery pm, pan- …creatic, portal vein; spv, splenic … and vein; spl, spleen, st,

… following drugs were used: acetylcholine chloride, L-adrenaline bitartrate, atropine sulphate, car-…prine chloride, 5-hydroxytryptamine creatinine sulphate, DL-isoprenaline hydrochloride, mecamylamine hydrochloride, nicotine sulphate, L-noradrenaline bitartrate, phentolamine methane sulphonate, phenylephrine hydrochloride, propranolol hydrochloride, salbutamol chloride. The amounts or concentrations of these drugs are expressed in moles or mol/l (M) respectively

…cent histochemistry

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TABLE I Affinity (pD_2) values for the different agonists tested on spleen strips and coeliac mesenteric artery strips from *Scyliorhinus canicula* and *Squalus acanthias*. The values are means ±S.E. Numbers within parenthesis indicate number of experiments.

	Adrenaline	Noradrenaline	Phenylephrine	Acetylcholine
Scyliorhinus				
Spleen	6.19 ± 0.09 (7)	5.59 ± 0.08 (16)	5.47 ± 0.05 (4)	no respons[illegible]
Arteries	6.75 ± 0.[illegible]2 (3)	5.95 (1)	—	5.96 ± [illegible]
Squalus				
Spleen	6.76 ± 0.06 (30)	6.11 ± 0.09 (14)	5.84 ± 0.11 (10)	4.97 ± [illegible]
Arteries	6.29 ± 0.17 (6)	5.37 ± 0.13 (14)	6.06 ± 0.17 (5)	6.03 ± [illegible]

= value calculated from only 5 dose-response curves out of 19 expts. (see text).

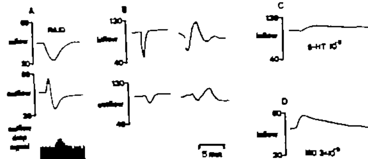
Cholinergic drugs Acetylcholine contracted the artery strips from both *Scyliorhinus* [illegible] *Squalus* (Table II Fig. 6). Spleen strips from *Scyliorhinus* did not visibly respond to [illegible]choline 10[illegible] – 10[illegible] M while *Squalus* spleen strips usually contracted both to acetylch[illegible] (Table I Fig. 6) and carbacholine (pD = 6.17 ± 0 18, n = 4). The contractions produ[illegible] acetylcholine, however was often weak and irregular and in only 5 expts. out of 19 [illegible] cumulative dose-response curves were obtained. The results from these 5 expts. [illegible] presented in Table I and Fig. 6.

Experiments with perfused spleen

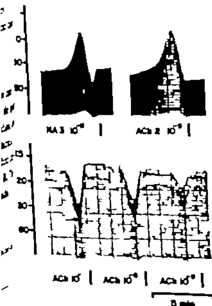
A perfusion pressure of 25–30 cm H_2O produced an initial flow rate of 0.6–0.7 m[illegible] (*Scyliorhinus*) or 1 0–2.5 ml/min (*Squalus*) respectively through the spleen. Inject[illegible] appropriate agonists into the perfusion stream in single doses produced either constr[illegible] or dilatation of the spleen. The constriction was manifested as a decrease of the flow on the inflow side, and a biphasic response due to expulsion of fluid from the spleen foll[illegible] by a decrease in flow rate on the outflow side (Fig. 2 A 3 A and 3 B). The dilatation [illegible] detected as a simple increase in flow on both the inflow and outflow sides (Fig. 2 C, 2 D 3 C).

TABLE II pA values for the effect of phentolamine on noradrenaline dose-response curves from [illegible]*liorhinus canicula* and *Squalus acanthias* spleen and artery preparations. Numbers within p[illegible]thesis indicate number of experiments.

	pA_2	pA_{10}		Dose range
Scyliorhinus				
Perfused spleen	6.7	5.7	(3)	10[illegible] – 3 10[illegible] M
Spleen strips	5.9	5.0	(3)	10[illegible] – 10[illegible] M
Squalus				
Perfused spleen	7.5	6.6	(4)	10[illegible] – 10[illegible] M
Spleen strips	6.7	5.7	(2)	10[illegible] – 10[illegible] M
Artery strips	5.9	4.8	(2)	10[illegible] – 10[illegible] M



Effects of drugs and nerve stimulation on the inflow and outflow of perfusion fluid in the dogfish spleen. *A. Scyliorhinus.* The inflow rate decreases in response to noradrenaline, while the outflow rate increases due to expulsion of fluid from the spleen and then decreases during splenic constriction. During the constriction erythrocytes are expelled into the venous effluent. The resulting increase in optical density of the venous effluent is detected by the dropcounter and recorded as an increased amplitude of the drop signal. *B. Squalus.* Effects of nerve stimulation with 15 Hz, 0.5 ms duration and 8V for 30 s: either a simple flow decrease on both the inflow and outflow sides (left), or a complex response with an initial increase due to expulsion of fluid, a period of spleno-constriction and a period of splenodilatation (right). The latter response was seen in few specimens only. *C. Squalus.* Splenodilatation produced by 5-hydroxytryptamine. *D. Scyliorhinus.* Low doses of isoprenaline produce spleno-dilatation as shown in the figure, while higher doses give responses mimicking those produced by noradrenaline. Numbers on vertical axes refer to the flow rates in drops per minute. All doses are expressed in moles.



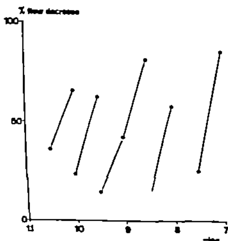


Fig. 3

Fig. 4

Fig. 3. *Scyliorhinus.* Effects of noradrenaline and acetylcholine on the outflow rate. *A* and *B* show the difference between the drugs in that noradrenaline causes an initial expulsion of fluid from the spleen. *C* shows the dilatatory effect of acetylcholine occasionally seen. In this case phentolamine (10^{-6} M) was present in the perfusion fluid. Note that with this drop recorder an increase in vertical size of the signal represents decrease in flow rate, and that the recordings go from right to left.

Fig. 4. Parallel shift in the dose-response curve for noradrenaline on the *Squalus* perfused spleen by phentolamine. From left to right: control, phentolamine 10^{-} 3 10^{-7} 10^{-6} and 3 10^{-6} M.

TABLE III Affinity (pD_2-) values for the different adrenergic agonists tested on perfused spleen preparations from *Scyliorhinus canicula* and *Squalus acanthias*. The values are given as mean. Numbers within parenthesis indicate number of experiments.

	Adrenaline	Noradrenaline	Phenylephrine	Isoprenaline
Scyliorhinus	10.97 ± 0.31 (4)	10.71 ± 0.22 (5)	10.08 ± 0.54 (4)	5.84 + 0.2 (4)
Squalus	9.96 ± 0.14 (10)	9 48 ± 0.10 (13)	7 86 ± 0.38 (5)	< 5 (4)

At the beginning of each experiment the splenic constriction was accompanied by expulsion of erythrocytes into the venous effluent, this effect being most pronounced in *Scyliorhinus*. The release of red blood cells is clearly seen as a reddening of the venous effluent; the resulting increase in optical density of the perfusion fluid is often detected as an increase in the amplitude of the outflow drop signal from the photocell (Fig. 2 A).

Adrenergic drugs A dose-dependent constriction of the spleen of both species was produced by adrenaline, noradrenaline and phenylephrine (Table III, Fig. 2 A and 3 A). Isoprenaline in lower doses (up to 3 10^{-9} mol) produced dilatation of the spleen (Fig. 2 D), dilatation being most pronounced in *Scyliorhinus* and more inconsistent in *Squalus*. High doses of isoprenaline caused splenic constriction in both species in a manner resembling other catecholamines and phenylephrine. Dilatation was also produced by 5-hydroxytryptamine ($10^{-9} - 10^{-6}$ mol) in both species (Fig. 2 C), and by salbutamol ($10^{-9} - 10^{-6}$ mol) in *Scyliorhinus*

Phentolamine competitively antagonized (Fig. 4) the constrictory effects of noradrenaline in both species (Table II). In *Scyliorhinus* a weak dilatation could be seen with noradrenaline after phentolamine (10^{-6} M).

Cholinergic drugs The effects of acetylcholine were in both species very inconsistent, and elucidation of the mechanism of action of this drug is further complicated by the fact that desensitization rapidly occurred during the experiment. The desensitization appeared to be manifested in two phases, one rapid initial phase and one second much slower phase during which some tentative conclusions of drug effects could be made.

In *Scyliorhinus* the most usual response to acetylcholine was a constriction of the spleen

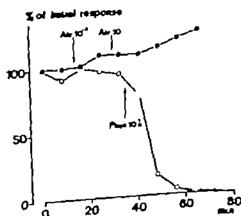
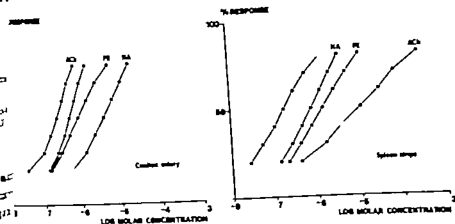


Fig. 5 Effects of atropine (●) or phentolamine (○) in reducing the splenic constriction caused by electrical stimulation of the nerves of *Squalus* for 30 s every 5 min at 15 Hz with pulses of 0.5 ms duration and 10 V



4 Dose-response curves in the interval 20–80 % of maximal response for each drug on isolated spleen (right) and coeliac artery strips (left) from *Squalus*. The curves show the mean individual sensitivity ... and Simmons 1961). A, adrenaline; NA, noradrenaline; PE, phenylephrine, ACh, acetylcholine. ...ould be noted that the dose-response curve for acetylcholine on the spleen strips is based only on results ... strips where proper dose-response curves could be obtained. No corrections for different maximum ... (intrinsic activity) has been made. For number of experiments on each drug consult Table 1.

...), within the range 8–10), but the initial flow increase on the outflow side due to expulsion ... fluid during the start of the constriction was not present unless high doses of acetylcholine ...re applied (Fig. 3 B). Phentolamine (10^{-4} M) abolished the initial flow increase caused by ...h doses of acetylcholine, and in two cases the constrictory response to acetylcholine was ...erted to a dilatation (Fig. 3 C). Occasionally acetylcholine exerted only dilatatory effects ...thout previous addition of alpha adrenoceptor blockers. Atropine (10^{-4}–2 10^{-4} M) ...mpletely blocked those constrictory effects of acetylcholine which are not subject to the ...ost rapid desensitization, and in two cases also the dilatatory response, although the effect ...as impossible to quantify due to desensitization.

In *Squalus* the normal response to acetylcholine was a constriction mimicking that caused ...y adrenaline. In one case a slight dilatation was produced by acetylcholine (10^{-4} mol), and ...n 3 expts. no response at all could be obtained. Phentolamine (10^{-} –10^{-4} M) reduced, ...although never abolished, the effects of 10^{-4} 10^{-4} mol of acetylcholine during the slower ...phase of desensitization. Mecamylamine (10^{-4} M) was without detectable effects on the ...acetylcholine response.

Nerve stimulation. Electrical stimulation of the vascular bundles to the spleen suggests that few if any sympathetic nerves pass to the spleen in *Scyliorhinus*. In 3 expts. out of 13 a very slight flow decrease was recorded in response to electrical stimulation with 20 Hz 0.5–1.0 ms duration and 3–18 V for 30–60 s. The responses were too small and inconsistent to allow further analysis.

In *Squalus*, however significant and reproducible changes in perfusion flow through the spleen occurred during stimulation of the splenic nerves following the lieno-gastric arter... (Fig. 2 B). Stimulation carried out with 0.5–1.0 ms duration, 10–20 Hz and 3–12 V for 30... with 8 min intervals ga... reproducible responses for at least 2 h. The flow change durin...

TABLE III. Affinity (pD_2-) values for the different adrenergic agonists tested on perfused spleen p[illegible]tions from *Scyliorhinus canicula* and *Squalus acanthias*. The values are given as mean [illegible] Numbers within parenthesis indicate number of experiments.

	Adrenaline	Noradrenaline	Phenylephrine	Isoprenaline
Scyliorhinus	10.97±0.31 (4)	10.71±0.22 (5)	10.08±0.54 (4)	5.94±0.[illegible]
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At the beginning of each experiment the splenic constriction was accompanied by [illegible]sion of erythrocytes into the venous effluent, this effect being most pronounced in *Scyliorhinus*. The release of red blood cells is clearly seen as a reddening of the venous effluent; the resulting increase in optical density of the perfusion fluid is often detected as an [illegible] in the amplitude of the outflow drop signal from the photocell (Fig. 2 A).

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Phentolamine competitively antagonized (Fig. 4) the constrictory effects of noradr[illegible] in both species (Table II). In *Scyliorhinus* a weak dilatation could be seen with nor[illegible] after phentolamine (10^{-6} M).

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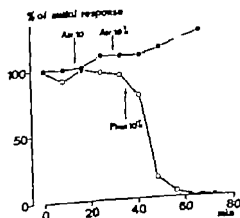
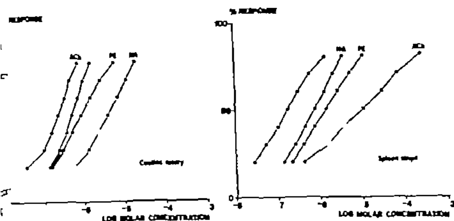


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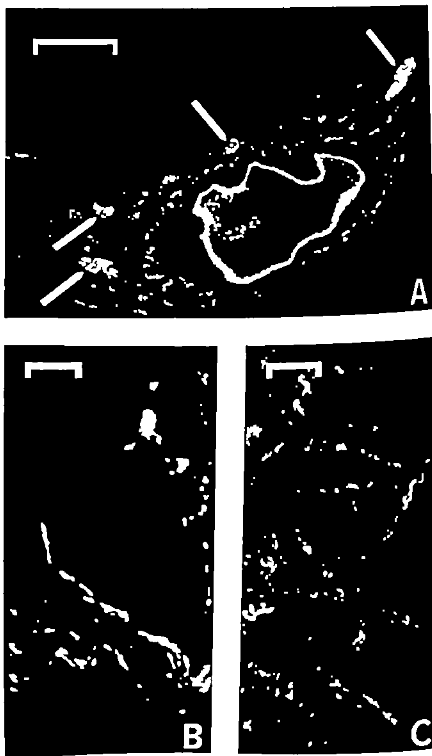


Fig. 7 *Squalus*, heno-gastric artery Formaldehyde treatment for 1 h. *A*. Di gonal section through artery wall showing a well developed innervation by fluorescent f bres. At arrows large bundles of fluorescent fibres following the vessel. Not the strongly utofluorescent inner elastic membrane. Cal tion 400 µm. *B* Bundle of varicose f bres the artery wall. Calibration 25 µm. *C* Longitudinal sec through the artery wall. Mesh-work of smooth and terminal fluorescent f bres. Background fluoresc due to autofluorescent connective tissue. Calibration 100 µm.

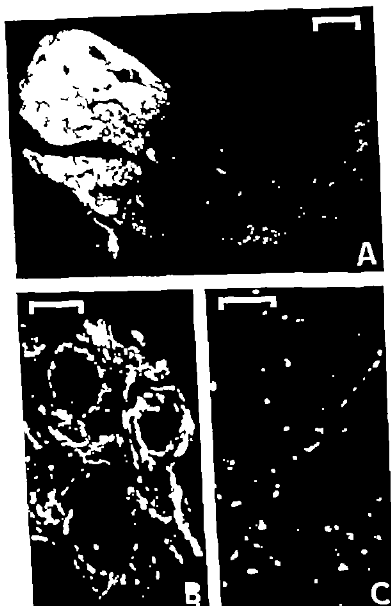


Fig. 8. *Squalus*. Sympathetic ganglion (*A*, *B*) and spleen (*C*). Formaldehyde treatment for 1 h. *A*. Section through one of the anterior sympathetic ganglia, the gastric ganglion, showing ganglion cells surrounded by fluorescent fibres and strongly fluorescent chromaffin tissue. To the right a bundle of nerve fibres leave the ganglion. Calibration 250 μm. *B*. Weakly fluorescent ganglion cells surrounded by brightly fluorescent fibres. Calibration 50 μm. *C*. Fluorescent varicose fibres in the spleen of an animal pre-injected with noradrenaline. Background fluorescence due to yellow and green cellular autofluorescence. Calibration 50 μm.

stimulation is sometimes polyphasic in both the inflow and outflow sides, including a pa of spleno-dilatation (Fig. 2 B). This splenodilatation occurs whether or not the dem stimulation is continued, and is thus not due to any "rebound phenomenon (Campbell Burnstock 1968)

The splenic constriction produced by nerve stimulation could be rapidly blocked phentolamine (10^{-7} M) but was unaffected or slightly enhanced by atropine (10^{-6} 10^{-5} Fig. 5) Mecamylamine (10^{-4} M) lacked detectable effects. Where a dilatation followed constriction during nerve stimulation this was also invariably abolished by phentola (10^{-} M) Nicotine caused a weak rapidly desensitized constriction in the *Squalus* sple

Fluorescent histochemistry

In the coeliac and lieno-gastric arteries characteristically bluish-green brightly fluoresc nerve fibres could be seen after treatment with formaldehyde vapour for 1 h (Fig. 7 A, B, C Large bundles of smooth fluorescent fibres could also be seen following both the lieno-gas (Fig. 7 A) and the coeliac arteries. Bright autofluorescence was seen in the inner ela membrane (Fig. 7 A)

Treatment for 1–3 h in formaldehyde vapour of spleen preparations from normal noradrenaline-preinjected animals produced but few fluorescent terminals in the spleen tiss except close to the small arteries (Fig. 8) The presence of strong yellow or green autofluo cence would obscure nerve fibres in most parts of the spleen

In sympathetic ganglia weakly but specifically fluorescent ganglion cells with surround terminals could be seen after 1 h formaldehyde treatment (Fig. 8) together with extend bright clusters of chromaffin cells (Fig. 8 A)

Discussion

Contrary to earlier reports (Opdyke and Opdyke 1971) the dogfish spleen in our experience is able to release erythrocytes when stimulated The release of red blood cells can clearly b observed to be smaller than in the cod (Nilsson and Grove 1974), and may therefore repres a less developed stage in this respect

The presence of alpha adrenoceptors, in both the spleen and arteries, mediating contra tion of the tissues is demonstrated in both species from the order of potency of the adrenoc agonists and the pA values for phentolamine It is interesting to note that in *Squalus* phe ylephrine has a higher pD than noradrenaline on the arteries, which may in part be due t noradrenaline and adrenaline effects simultaneously on beta adrenoceptors. The parall shift of the noradrenaline dose response curve produced by phentolamine in all preparations tested, indicates that this drug acts by competitively blocking the alpha adrenoceptors of th smooth muscles.

The unpredictable nature of the dilatation produced by isoprenaline did not allow prop tests with beta adrenoceptor antagonists, so we can only suggest that it is due to a beta adreno ceptor mechanism since noradrenaline (after phentolamine) and salbutamol also had wea dilatatory actions.

The variability of the muscarinic reaction to acetylcholine may depend on simultaneou

...ions of trabecular, capsular and intrinsic blood vessel smooth muscle cells. It is possible, ...ple, for contraction of certain trabeculae to enlarge the local blood space. In the ...ce of detailed knowledge of the sites of action of acetylcholine, particularly in *Scylio*...s which has little splenic innervation, an explanation of the observed acetylcholine ...s must await a more detailed study of blood flow through the spleen of these fish.

...e part of the acetylcholine response which is subject to the most rapid desensitization ... in part be due to release of catecholamine (in *Squalus*). This may be noradrenaline, ...h is the dominating catecholamine in several organs in this animal (Euler and Fänge ...1).

...oth the constrictory and dilatatory effects in the polyphasic response to electrical stimula-... sometimes seen in *Squalus* can be rapidly blocked by phentolamine in relatively low ...centrations, and this may be taken to indicate that the nerve effects are ultimately media-... by an adrenergic transmitter. Fluorescent histochemistry shows few adrenergic nerve ...inals in the spleen and the innervation may regulate mainly the vessels. It is also possible ...t the bright autofluorescence obscures specifically fluorescent nerve terminals on the spleen ...scle cells.

...n *Scyliorhinus* the innervation of the spleen seems to be poorly developed as judged from ...k or absent responses to electrical stimulation of the vascular bundle. Regulation of the ...ce and release of erythrocytes in this species may largely depend on the levels of circulat... adrenaline and noradrenaline in the blood. Mazeud (1969) found in the 'normal' *Scylio*...us blood plasma 2.4 µg adrenaline and 4.8 µg noradrenaline per 100 ml plasma, which in ...mals kept in air for 30 min was increased to 8.5 and 13.8 µg per 100 ml plasma respec-...ely. These values compare closely with the observed concentration ranges over which ... effects of adrenaline and noradrenaline occur in isolated artery and spleen strips. (In ...*yliorhinus* ED_{50} for spleen strips 11.8 and 42.3 µg/100 ml and ED_{50} for artery strips 3.6 ...d 16.9 µg/100 ml for adrenaline and noradrenaline respectively.) Such changes in the ...techolamine concentration in the blood plasma may therefore control both the spleen and ... arteries. Davies and Rankin (1973) similarly concluded that circulating catecholamines ...ay an important role in controlling the branchial circulation in *Scyliorhinus*. By the same ...gument, circulating catecholamines will reinforce the sympathetic innervation of the ...ysalus spleen.

...he work ...as supported by the Swedish Natural Science Research Council and the Knut and Alice Wallen-...rg Travelling Fund

References

...ARENS, E. J. and A. M. SIMONIS, Analysis of the action of drugs and drug combinations. In: *Quantitative methods in pharmacology.* Ed. by H. de Jonge, North Holland Publishing Comp., Amsterdam 1961. 284–311.

...OWEY, B. G. and S. A. GRILLO, Antagonism of acetylcholine by adrenaline antagonists. *Brit. J. Pharmacol.* 1963. 20. 528–533.

...OYNET, V. De l'influence de l'hémorragie et de l'asphyxie sur le nombre des hématies dans le sang circulant des vertébrés inférieurs. *J. Physiol. Path. Gén.* 1929. 27. 735–740.

...AMPBELL, G. and G. BURNSTOCK, Comparative physiology of gastrointestinal motility. In: *Handbook of Physiology.* Vol IV. Section 6. Alimentary canal: Motility. Ed. by C. F. Code, American Physiological Society. Washington DC. 1968. 2213–2266.

...

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The variability of the muscarinic reaction to acetylcholine may depend on simultaneo...

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Studies of Some Twitch and Fatigue Properties of Different Motor Unit Types in the Ankle Muscles of the Adult Cat

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Abstract

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…nractile responses of motor units in the gastrocnemius, soleus and pretibial flexor muscles of adult … were elicited by intracellular stimulation of motoneurones. The motor units were classified into types … FR and S (Burke et al. 1973) and their responses to the same stimulation patterns as those used in … vious investigation of whole muscles (Hammarberg and Kellerth 1975 a) were studied. The duration … motoneurone afterhyperpolarization was short in both the fast twitch FF and FR units. It was longer … in soleus S units than in the S units of the pale muscles. Twitch time-to-peak was less than 30 ms in FF and FR units, but exceeded 40 ms in the S units. Soleus S units were slower than S units of the pale …cles. Potentiation was observed in the gastrocnemius units, but not in the soleus S units. A short rest …ed fatigued extensor units of the FF and FR types to regain some contractile strength. This was less …dent in the S units which, on the other hand, were extremely resistant to fatigue. Differences in response …terns between corresponding motor unit types of the flexor and extensor muscles were observed. A few twitch units were identified in the slow soleus muscle.

…a previous investigation (Hammarberg and Kellerth 1975 a) the postnatal development … certain twitch and fatigue properties of the medial gastrocnemius, soleus and anterior …bial muscles of the cat was studied. In order to better understand the postnatal changes … motor function it was necessary to extend the investigation to the level of single motor …nits as well, since the performance of whole muscles reflects the summated activity of a …rge number of motor units which are known to display differing functional properties (Devanandan et al. 1965, Henneman and Olson 1965, McPhedran et al. 1965, Wuerker et al. 1965, Burke et al. 1971, 1973, 1974, Olson and Swett 1971, Goslow et al. 1972, Mosher et al. 1972, Bagust et al. 1973, Bagust 1974, Stephens and Stuart 1974).

In the present study different types of motor units of the ankle muscles of the adult cat have been investigated with respect to their responses to the same functional tests as those applied in the previous study of whole muscles (Hammarberg and Kellerth 1975 a). In addition to the twitch and fatigue properties of the muscle fibres, the duration of the post-spike after

DAVIES, D T and J C. RANKIN, Adrenergic receptors and vascular responses to catecholamines [illegible] dogfish gills. *Comp. gen. Pharmacol* 1973. *4* 139–148.

EULER, U S. and R FÄNGE, Catecholamines in nerves and organs of *Myxine glutinosa*, [illegible] and *Gadus callarias*. *Gen. comp Endocr* 1961 *1* 191 194.

FALCK, B. and CH. OWMAN A detailed methodological description of the fluorescence method [illegible] cellular demonstration of biogenic amines. *Acta Univ Lund.* 1965. *Sect. II No 7*

HOLMES, W N and E. M DONALDSON, The body compartments and distribution of electrolytes [illegible] *Physiology* Ed. by W S. Hoar and D J Randall, Academic Press, New York and London [illegible] 1–89

HOLMGREN, S. and S. NILSSON, Effects of some adrenergic and cholinergic drugs on isolated [illegible] from the cod, *Gadus morhua*. *Eur J Pharmacol.* 1975 *32.* 163–169

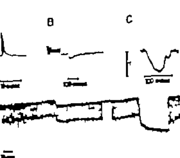
MAZEAUD, M., Influence de stress sur les teneurs en catecholamines du plasma et des corps [illegible] un Selacian, la Roussette (*Scyliorhinus canicula* L.) *C. R Soc. Biol.* (Paris) 1969. *163.* [illegible]

NILSSON S. and D J Grove, Adrenergic and cholinergic innervation of the spleen of the cod, [illegible] *Eur J Pharmacol.* 1974 *28* 135–141

OPDYKE, D F and N E. OPDYKE, Splenic responses to stimulation in *Squalus acanthias*. [illegible] 1971 *221* 623–625

ROSSUM, J M VAN, Cumulative dose-response curves. II. Technique for the making of dose [illegible] curves in isolated organs and the evaluation of drug parameters. *Arch. int Pharmacodyn.* [illegible] 299–330.

VAIREL, J Action de l adrénaline et de l'acétylcholine sur la rate. *J Physiol. Path. gén.* 1933. [illegible]



A B C D

Fig. 1

Fig. 2

Motor unit of the S type belonging to the MG muscle. *A* Motoneurone action potential. *B*. …urone AHP following an action potential elicited by intracellular stimulation. *C*. Twitch contraction … muscle unit. *D* Contractile responses of the muscle unit to the sequentially occurring "fatigue …" and "tetanus tests. In *C* and *D* as well as in the following figures, an increase in contractile … is signalled by downward deflection in the myogram.

… Motor unit of the S type belonging to the SOL muscle. *A*. Motoneurone action potential. *B*. …urone AHP following an action potential elicited by intracellular stimulation. *C*. Twitch contraction … muscle unit. *D* Contractile responses of the muscle unit to the sequentially occurring "fatigue … and "tetanus tests

…t be compared directly with those of Burke *et al* (1971, 1973), however since the latter authors …ed maximal post-tetanic potentiation in their units prior to the "fatigue test …th the result that the …mal tension output always occurred during the first contraction of the sequence. In the present study …maximal response sometimes did not occur until 15–20 after the onset of the "fatigue test" …rke *et al* (1971 1973, 1974) also stimulated their motor units …th pulse trains of 2 duration, each … interval corresponding to 125 of the contraction time of the unit. This mode of stimulation produced …sed tetanus and was used by Burke and his associates to separate the two fatigue resistant unit types …R (fast contracting, fatigue resistant) and "S" (slow contracting, fatigue resistant). The so-called … test" was used only in the earlier part of the present study since it was discovered that FR and S …s could also be separated by means of the "pause" and "tetanus tests"

Results

…e results presented here were obtained from 116 motor units belonging to the MG (52 …its), LG (36 units), SOL (17 units), TA (3 units) and EDL (8 units) muscles of adult cats.

S units of triceps surae Fig. 1 and 2 show typical examples of the behaviour of S units … the MG LG and SOL muscles, respectively during the various tests. *A* of Fig. 1 and 2 …ows the action potentials, while *B* illustrates at slower sweep speed the afterhyperpolarizations (AHP). Generally the AHP's of the SOL motoneurones were of longer duration …an those of the S units of the MG + LG muscles (p 0.002, Table 1). *C* of Fig. 1 and 2 …ow the twitch contractions elicited by intracellular stimulation of the motoneurones. …he mean value for twitch contraction time (time-to-peak) was considerably greater for the …leus S units than for the S units of gastrocnemius ($p < 0.001$) although there was some …verlap of values between the two populations (Fig. 8 and Table I). Also the mean values …or twitch half-relaxation time (HRT) were found to be different for the two groups of S …nits (p 0.05 Table 1).

D of Fig. 1 and 2 illustrates the contractile responses of the S units of the gastrocnemius

hyperpolarization (AHP) of the innervating motoneurones has also been investigated; this parameter had earlier been shown to differ between motoneurones supplying fast and slow twitch muscle fibres, respectively (Eccles *et al* 1958, Kuno 1959). The correspon... properties of immature motor units at various stages of postnatal development will be described in a following report (Hammarberg and Kellerth 1975 b).

Methods

Adult cats were anaesthetized with an intraperitoneal injection of Nembutal (40 mg/kg); additional amounts of anaesthetics, if needed, were administered during the course of the experiment through a cannula inserted in the left femoral vein. The medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (SOL), anterior tibial (TA) and extensor digitorum longus (EDL) muscles of the right hind limb were carefully dissected free without damage to their blood or nerve supplies. The corresponding tendons were cut and arranged fo separate attachment to a strain gauge myograph through short braided suture (compliance 0.05 mm/kg cm). The moto nerves to the MG LG+SOL and TA+EDL muscles were freed from surroundi g tissues and mounted intact on stimulating electrodes. The remaining portions of the ... tibial and common peroneal nerves were cut or crushed

After a lumbosacral laminectomy the ipsilateral dorsal roots L6–S2 were transected. The animal was placed in a rigid steel frame and immobilized along the spinal column, pelvic girdle and right hind limb. Exposed tissues were covered with paraffin oil. The rectal and muscle temperatures ... maintained at 35–38°C by means of infrared l ght. During the course of the experiment the animals ... allowed to breathe spontaneously through a tracheal cannula, except in the few cases where it ... necessary to perform a pneumothorax in order to decrease respiratory movements. In such cases artificial ventilation was ... In all animals expired CO was monitored continuously since hyper or hypoventilation have been shown to markedly influence the fatigue properties of muscles (Hammarberg and Kellerth 1975 a). Single barrel glass micropipettes with t p diameters of 1 μm were used for intracellular recording and stimulation of si gle spinal motoneurones. The microelectrodes were filled either with M potassium citrate (... resistance 3–8 MΩ), or more often w th a 5 solution of the dye Procion Yellow in distilled ... (... resistance 10–15 MΩ), since ne of the ims of the present experiments was to stain physiologically id... motoneurones for later ltrastructural analysis Following impalement each motoneurone was identified by antidromic stimulation of the muscle nerves Its appropriate muscle as then connected to the strain gauge, and contractile responses of the muscle f bres innervated by it ere elicited by intracellular stimulation of the motoneurones (depolarizing pulses of 2 ms duration). For each motor unit the passive ... of the muscle was adjusted to give maximal tw tch responses I all cases, however the amount of ... stretch was less than that correspondng t 90° dorsiflexion of the ankle. During the course of the experiment, regular checks were made of the tw tch tensions of the whole muscles. If a reduction ... occurred, indicating a deterioratio of the muscle, the experiment was terminated The electrical and mechanical activities of the mot units were di played on an oscilloscope and by a dc-coupled pen ... (Devices M2).

Testing procedures

Immediately follow g the impalement of a motoneu ne, its membrane potential and antidromic ... potential were reco ded However since these ... were probably often distorted due to high electrode resistances, they were used o ly for continuo monit ring of cell condition dur g the time of impalement. The afterhyperpolarization (AHP) follow g act ... potentials evoked by intracellular stimulation ... studied.

The contractile responses of muscle f bres belo ging t single motor units were studied both ... single pulse stimulation and during the different modes of repetitive stimulation described in the previous study on whole muscles, *i.e.* the "fatigue ... "pause and "tetanus tests (H mma berg and Kellerth 1975 ...). In the latter I estigation the pause test was initiated both before and after the "tetanus test" ... the first of these two "pause tests has been considered ... the present study since erroneous results due to cell deterioration are more liable to appear w th p olo ged periods of microelectrode impalement. The "f tigue index of un t was calculated as the ratio of the amplitude of the 120th contraction of the "f tigue test to that of the first contraction of the sequence. The "fatigue indices of the present ...



Fig. 3

Fig. 4

i. Motor unit of the FR type belonging to the LG muscle. *A*. Motoneurone action potential. *B*. neurone AHP following an action potential elicited by intracellular stimulation. *C*. Twitch contraction muscle unit. *D*. Contractile responses of the muscle unit to the sequentially occurring "fatigue" e" and "tetanus tests". *E*. Contractile response to unfused tetanus stimulation (the "sag test").

i. Motor unit of the FF type belonging to the MG muscle. *A*. Motoneurone action potential. *B*. neurone AHP following an action potential elicited by intracellular stimulation. *C*. Twitch contrac of the muscle unit. *D*. Contractile responses of the muscle unit to the sequentially occurring "fatigue se" and "tetanus tests".

units of triceps surae

3 shows response pattern typical of FR units. *In general the AHP's of these units* of shorter duration than those of the S type (p < 0.01 Fig. 8, Table I). The mean for time-to-peak of twitch contractions was also considerably less for FR units *n for the S units (p 0.001), and in no case did the contraction time exceed 30 ms* 8, Table I). *E* of Fig. 3 shows the muscle response to unfused tetanus, where the pres- of sag further separates the FR units from the S type of units (Burke *et al.* 1971 3, Proske and Waite 1974).

D of Fig. 3 shows contractile responses during the "fatigue" "pause" and "tetanus ts" By definition, the "fatigue index" of an FR unit (Fig. 3) is > 0.75 In the present dy "fatigue indices up to 1 67 were encountered (see also Stephens and Stuart 1974). In extensor units of the FR type, the 10 s pause resulted in a significant (p < 0.001) increase contractile strength when stimulation was resumed (Fig. 7 Table I). In some cases, the sion developed immediately after the pause even exceeded that produced earlier during "fatigue test revealing most likely a potentiating effect of the iterative stimulation Olson and Swett 1971). *In contrast to the S units (Fig. 1 D and 2 D), the FR units* owed no immediate change in tension output with the onset of the tetanus (Fig. 3 *D*), the t/p tension ratio was close to 1.0 (Fig. 7 and Table I). However, during the course the tetanus the contractile tension of typical FR units gradually increased (24.4 ± 29.1 %), d this effect even remained for some time after the iterative stimulation had been resumed ig. 3 *D*). From the responses of the FR and S units it may therefore be concluded that y using the same tests which had previously been applied to study differences in fatiguability mong whole muscles (Hammarberg and Kellerth 1975 a) it is also possible to separate xtensor FR and S units.

TABLE I. A summary of observations of 105 motor units belonging to the triceps surae muscle. For each parameter the mean value ±SE has been indicated. The total range of [illegible] is given in parenthesis. Because of the large relative error introduced when trying to measure the small often negligible, contractile tensions produced by the FF units during the "pause" and "tetanus tests" the corresponding values have been omitted in the Table.

Motor unit type	Duration of motoneurone AHP (ms)	Contraction time (ms)	HRT (ms)	Fatigue index	Pause effect (%)	[illegible]
FF (n = 46)	110±36 (50–190)	18.3±3.[illegible] (11–27)	13.1±4.9 (8–23)	0.07±0.06 (0.00–0.[illegible]5)	—	—
FR (n = 21)	101±26 (70–160)	20.9±4.1 (16–29)	20.5±5.1 (1[illegible]–28)	0.97±0.16 (0.77–1.67)	20.2±4.1 (15–30)	1.[illegible] (1.[illegible])
S (n = 9)	136±25 (90–190)	49.1±12.6 (40–75)	53.8±15.3 (36–80)	1.06±0.11 (0.98–1.[illegible]8)	4.0±3.5 (0–10)	1.[illegible] (1.[illegible])
Soleus S (n = 13)	198±30 (145–250)	75.4±13.6 (52–95)	77.3±[illegible]6.6 (36–110)	0.94±0.06 (0.85–1.00)	[illegible]5±[illegible]9 (0–9)	1.[illegible] (1.[illegible])
Unclassified (n = 16)	105±27 (65–135)	[illegible]2.1±4.8 (13–29)	16.6±5.9 (10–2[illegible])	(0.[illegible]5–0.75)	27.8±14.6 (12–53)	1.00–1.[illegible]

and soleus muscles during prolonged activation. Each record begins with a "fatigue test" lasting for 2 min, followed by a 10 s pause. Iterative stimulation was then resumed, but again interrupted after approximately another 30 s by the "tetanus test". The S units are very resistant to fatigue and, by definition, develop at least 75% of their initial contraction tension after 2 min of the "fatigue test", *i.e.* they have a "fatigue index" > 0.75 (Burke et al. 1971). In the present study the soleus S units had "fatigue indices" which were smaller than those of the gastrocnemius S units (p < 0.05, Table I). The changes in contractile tension following the "pause test" were quite insignificant in all cases, even for those S units which had displayed the lowest values for fatigue index.

Immediately with the onset of the "tetanus test" all S units developed a tension which exceeded that of the last contraction of the preceding "fatigue test" by more than 15 per cent (Fig. 1 and 2), *i.e.* they had tetanus/pretetanus (t/p) tension ratios greater than (Fig. 7 and Table I). Furthermore, the S units maintained their tension throughout the period of tetanic stimulation and exhibited no signs of fatigue. Sometimes the S units of MG+ but *not* those of SOL, even showed a gradual increase in tension (7.4±10.2 %) during tetanus (Fig. 1). This difference (p < 0.05) between the S units of the two muscles possibly be accounted for by a difference between them in potentiation (Olson and S 1971, Burke *et al.* 1974). This possibility is supported by the increased amplitude of contractions immediately following the tetanus of the S units of MG + LG (Fig. 1), as well as by the observed tendency for these units to have a somewhat larger "fatigue index" (p Table I). In summary the S units of MG + LG and SOL are similar with respect to responses to the various modes of repetitive stimulation, even though the units of the former muscles seem to be more potentiated, but there are also clear dissimilarities between them in their mean values for contraction time and AHP duration (Fig. 7 and 8, Table

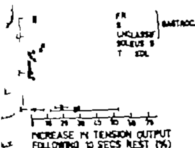


Fig. 7

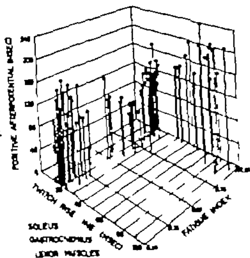


Fig. 8

Graphical illustration of the relation between the t/p tension ratio (ordinate) and the "pause effect" ...) for extensor and flexor motor units of different types.

Three dimensional plot showing the interrelation between the motoneurone AHP (vertical Y-axis), ... rise time-to-peak (horizontal X-axis) and the "fatigue index" (horizontal Z-axis) for motor units ... ing to the SOL, MG LG, and TA EDL muscles.

... the FF units (p 0.05) and smaller than that of the FR units (p<0.1 Table I). With ... ect to their responses to the "fatigue and "tetanus tests" the "unclassified" units at ... e times resembled the FR units (Fig. 5), and at other times they more closely resembled ... FF type of units (Fig. 6). The average decrease in tension during the course of the ... ... test amounted to 89.7±9.9%. The pause test usually caused a somewhat ... er recovery in contractile tension in the "unclassified" units than in the FR units ... ble I).

... tor motor units

... pite considerable effort only 11 motor units belonging to the TA (3) and EDL (8) muscles ... e successfully studied in the present investigation. Ten of these units had contraction ... es varying between 16 and 24 ms (mean value 20.8±2.5 ms) and were assigned to the ... (3 units), FR (5 units) and "unclassified (2 units) types on the basis of their responses ... the "fatigue test One fatigue resistant unit with contraction time of 36 ms was classi... ... as type S unit, since it did not "sag (Burke *et al.* 1973). The duration of motoneurone ... IP of the fast twitch units varied between 65 and 110 ms (mean value 89.0±14.7 ms), ... ile the AHP duration of the type S unit was 155 ms.

Although it is not possible on the basis of the small number of units studied here to draw ... y definite conclusions, it is our impression that with regard to the responses to repetitive ... mulation, the flexor units present picture which is somewhat different from that of the ... tensor units (Fig. 7 see also Hammarberg and Kellerth 1975 b). For example, the flexor ...

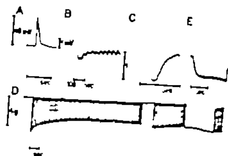


Fig. 5

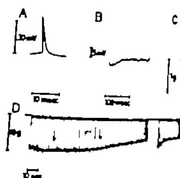


Fig. 6

Fig. 5 Unclassified motor unit belonging to the SOL muscle. *A* Motoneurone action Motoneurone AHP following an action potential elicited by intracellular stimulation. C Twi tion of the muscle unit. *D* Contractile responses of the muscle unit to the sequentially occurrin "pause" and "tetanus tests

Fig. 6. Unclassified" motor unit belonging to the LG muscle. *A*. Motoneurone action p Motoneurone AHP following an action potential elicited by intracellular stimulation. C Twi tion of the muscle unit. *D* Contractile responses of the muscle unit to the sequentially occurring "pause and "tetanus tests

FF units of triceps surae

Fig. 4 illustrates the typical behaviour of an FF" (fast contracting, fast fatigue) u AHP's of the FF units were generally of durations of the same magnitude as those FR units. The two unit types were also quite similar with respect to twitch contracti (Table I), although the fastest twitches encountered in the present study (contractio ~11 ms) were found among the FF units. The mean values for HRT on the othe were found to be significantly different ($p < 0.001$) for the FF and FR units (Table I

During the "fatigue test" (Fig. 4 *D*) a nearly complete extinction of FF unit re after 2 min of stimulation was not unusual, but any fatigue index within the limits d for FF units, *i.e.* <0.25 might be encountered The 'fatigue test resulted either in a s decline in contractile tension or more often, in an initial decrease in tension tempo interrupted by an increase before the final prevalence of the fatigue (Fig. 4 *D*). The effect was possibly due to the interaction of two physiological phenomena evoked by titive stimulation, namely fatigue and potentiation (cf von Euler and Swank 1940) pause test" usually allowed the units to retain some tension for 5–10 s (Fig. 4 *D*) absolute values of contractile tension being quite small however both before and afte test. Those FF units in which some contractile tension still remained 30 s after the "p test" were immediately exhausted when the tetanus test" was commenced (Fig. 4 *D*) no case did the t/p tension ratio exceed 1 00

"Unclassified" units of triceps surae

Sixteen of the 105 units studied in triceps surae had "fatigue indices between 0.25-0 and were therefore regarded as "unclassified" units (Burke *et al* 1973). All of these u had fast twitches and the AHP durations were not significantly different from those of t FF and FR units (Fig. 8 Table I). The mean value for HRT however was larger than th

…hese units were submitted to the same series of tests as the extensor motor units, the …he patterns obtained were quite different in certain respects. So far then, an identity …t be established between the motor unit types of triceps surae and the pretibial flexors, … studies are needed to investigate this problem (*cf* Mosher *et al.* 1972). Similar …were also obtained in studies of kitten motor units (Hammarberg and Kellerth …).

…pike afterhyperpolarization (AHP)

…resent study confirms the observation that motoneurones supplying the slow twitch …muscle are generally characterized by more long-lasting AHP's than motoneurones …k muscles (Eccles *et al.* 1958, Kuno 1959). However the *S units of the pale muscles* …ad AHP's of long duration, although not as long as those of the *SOL units. No sig-* …*nt difference in* AHP duration was found to exist between FF FR and "unclassified" … The absolute values for AHP's were found to be considerably greater than those re-…d by several previous investigators (Kuno 1959 Burke 1967 Proske and Waite 1974), …ere generally consistent with those reported by Eccles *et al.* (1958). A possible expla-… for this may be that Eccles *et al.* (1958), like ourselves, measured AHP duration to …mount of the terminal after-depolarization which *follows the AHP (Lloyd 1951)*, while …ther investigators measured the AHP to the point where it passed from hyperpolariza… to the initial level. One would expect the contribution of recurrent Renshaw-inhibition …HP to be negligible in the present study since, first, this inhibition is usually of shorter …tion than the AHP (Renshaw 1946) and, second, action potentials were elicited by intra-…lar stimulation of single neurones rather than by massive antidromic activation of whole …oneurone pools.

…ch contraction

…r differences in mean values for both contraction time and HRT were observed between …gastrocnemius FF FR "unclassified" units, the gastrocnemius S units and the soleus …nits. Furthermore, the HRT mean values were also significantly different between the … and FR types of units. In no case did the contraction time of a fast twitch unit exceed …ms, and in no case was the corresponding value for an S unit less than 40 ms. Burke …57) also observed *that* no motor units in triceps surae had contraction times between … and 40 ms and Proske and Waite (1974) found that the contraction time separating the … (FF and FR) and slow (S) units was about 40 ms. In general, the contraction times …rved in the present study were considerably shorter than those reported by Burke *et al.* …73 1974), and somewhat shorter than those reported by Proske and Waite (1974), but …re quite consistent with the values reported by Burke (1967) and Bagust (1974). Although …ferences in muscle temperature might explain in part these differing results (Gordon and …llips 1953 Buller *et al.* 1968), it should also be pointed out that in the *study by Burke* …*al.* (1973) the contraction times were measured on *maximally potentiated twitches*, which …ty have affected the results. In fact, Proske and Waite (1974) found that regardless of …otor unit type the contraction time for the majority of units increased by at least 50% after …olonged stimulation.

gible increase in contractile tension following the "pause test" and they decreased ... by 22.0 ± 15.0% during the course of the "tetanus test". Such a behaviour would ... expected from any of the extensor types of units (Table I). In addition, the flexor ... unit had a t/p tension ratio of only 1.00 which was never encountered among the ... units of the extensors (Table I).

Discussion

Motor unit types

In confirmation of the findings of Burke *et al.* (1973, 1974) this study found that by ... the "fatigue" and "sag tests" the majority of motor units in triceps surae could be di... into the 3 types FF, FR and S. It was also found that each of these motor unit types sh... a typical response to the "tetanus" and "pause tests". A minority of the motor units ... could not be assigned to any of the 3 major types mentioned above, and were consid... "unclassified" (Burke *et al.* 1973). All these "unclassified" units displayed AHP dura... contraction times and responses to the "pause test" which were quite similar to thos... the FF and FR unit types. With respect to their responses to the "fatigue" and "te... tests" however, they appeared to form a more or less continuous spectrum betwee... latter two types, in some cases more closely approaching the FF type, in other cas... sembling more closely the type FR units. We are therefore prone to agree with the c... clusion of Burke *et al.* (1973) that the fast twitch muscle units of triceps surae are lik... constitute a single population although the graded differences between them appear ... distributed in a bimodal pattern.

It has repeatedly been suggested on both physiological and histochemical grounds ... the slow motor units of the soleus may not be equivalent to the type S units found i... fast muscles (Nyström 1968 b, Mosher *et al.* 1972, Burke *et al.* 1974, Burke and T... 1974, Hammarberg 1974). The present study found the S units of both muscles to be ex... tremely resistant to fatigue. However, significant differences existed between them ... the soleus S units had both longer contraction times, HRT's and AHP durations tha... those of the gastrocnemius, and the behaviour of gastrocnemius S units during the "te... test" suggested a stronger potentiating effect operative in those units than in the S unit... the soleus (cf. Olsson and Swett 1971, Burke *et al.* 1974, Hammarberg and Kellerth 19... The present findings do not decisively indicate whether these differences between S unit... different muscles are qualitative rather than quantitative.

All thirty of the SOL motor units in the study of Burke *et al.* (1974) were classed as ... S. In the present investigation about fifty soleus motoneurones were impaled, but ... these, all of them innervating slow twitch muscle fibres were lost before all parameters h... been recorded. Of the remaining seventeen motor units four were found to display al... characteristics typical of fast twitch units (Fig. 5 and 8). Fast contracting SOL motor u... have been reported earlier (Fig. 4 of Bagust 1974) and may possibly be related to that sm... number of fibres with atypical histochemical staining properties which have been repor... to exist in the cat soleus muscle (see *e.g.* Ogata 1958, Hammarberg 1974).

Only a limited number of motor units of the flexor muscles were studied in detail, b...

...rison of results from single motor units and whole muscles

...the various tests used here to study single motor units have also been applied to whole ...s in a previous investigation (Hammarberg and Kellerth 1975 a), the relative contri-...n of various motor unit types to the behaviour of their parent muscles may be com-...d upon. As would be expected from its homogeneous histochemical composition ...rom 1968 b, Burke *et al.* 1974 Hammarberg 1974), the whole SOL muscle responded ...re or less the same way as the constituent units. The mean values for contraction time ...quite similar for the whole muscles (72 ms) and the single units (75 ms), as were also ...ean values for HRT (72 ms and 77 ms, respectively), "fatigue index" (0.89 and 0.94 ...ctively), t/p tension ratio (1.23 and 1.26, respectively) and "pause effect" (5.6% and ...respectively).

...r the gastrocnemius and pretibial flexors the mean values for contraction time of the ...e muscles were 24 ms and 22 ms respectively while the corresponding mean values for ...st twitch units of the same muscles were 20 ms and 21 ms, respectively. The gastrocne-... muscle had a moderately low mean value for "fatigue index" (0.71), was usually ...ntiated during the "tetanus test" and showed some recovery during the "pause test ...n also 15%). The anterior tibial muscle, on the other hand, displayed greater suscept...y to fatigue (mean value for "fatigue index" 0.49), showed no signs of potentiation ...ng the "tetanus test" and exhibited a marked recovery following the "pause test ...n value 29%). These findings are compatible with the histochemical observations of ...gher FR fibre content and a smaller proportion of FF fibres in the gastrocnemius muscle ...ompared to the tibialis anticus (Hammarberg 1974). As pointed out earlier however ... must also consider the possibility that the motor unit types of the flexor and extensor ...cles may not exhibit identical responses to the various tests employed.

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References

...ort J. Relationships between motor nerve conduction velocities and motor unit contraction characteristics in slow twitch muscle of the cat. *J. Physiol.* (Lond.) 1974. 238. 269–278.

...ort, J., D. M. Lewis and J. C. Luck, Post-tetanic effects in motor units of fast and slow twitch muscle of the cat. *J. Physiol.* (Lond.) 1974. 237. 115–121.

...ort J., S. Knott, D. M. Lewis, J. C. Luck and R. A. Westerman, Isometric contractions of motor units in fast twitch muscle of the cat. *J. Physiol.* (Lond.) 1973. 231. 87–104.

...ller, A. J., K. W. Ranatunga and J. M. Beith, The influence of temperature on the contractile characteristics of mammalian fast and slow twitch skeletal muscles. *J. Physiol.* (Lond.) 1968. 196. 82 P

...rke, R. E. Motor unit types of cat triceps surae muscle. *J. Physiol.* (Lond.) 1967. 193. 141–160.

...rke, R. E. and P. Tsairis. The correlation of physiological properties with histochemical characteristics in single muscle units. *Ann. N.Y. Acad. Sci.* 1974. 228. 145–160.

...rke, R. E., D. N. Levine, M. Salcman and P. Tsairis, Motor units in cat soleus muscle: Physiological, histochemical and morphological characteristics. *J. Physiol.* (Lond.) 1974. 238. 503–514.

...rke, R. E., D. N. Levine, P. Tsairis and F. E. Zajac, III, Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J. Physiol.* (Lond.) 1973. 234. 723–748.

...rke, R. E., D. N. Levine, F. E. Zajac, III, P. Tsairis and W. K. Engel, Mammalian motor units: Physiological-histochemical correlation in three types in cat ...

Fatigue properties

The change in contractile tension which occurs in a muscle or motor unit as a of repetitive stimulation may not depend solely upon the fatigue properties of the un also upon potentiation (von Euler and Swank 1940). This has important implica the interpretation of fatigue indices. Burke *et al* (1973) found both the FF and FR u be potentiated by repetitive stimulation. Olson and Swett (1971) on the other hand, a considerable difference between their two types of fast contracting units, the one v probably corresponded to the FF type showing little or no potentiation.

In the present investigation the "tetanus test" caused a visible potentiation of t not of the FF type unit. In the latter case, however an existing potentiation may hav concealed by the heavy fatigue component since signs of potentiation were usually the early phase of the "fatigue test" (Fig. 4) The motor units of the SOL muscle are to be little potentiated in comparison with the fast contracting units (Standaert 19 ström 1968 a, Olson and Swett 1971 Burke *et al* 1974). The "fatigue test" would be expected to reflect the pure fatigue component more accurately in the SOL moto than in the other motor units. This fact should be kept in mind when comparing "f indices" of different motor unit types.

The t/p tension ratio was found to differ considerably between fast twitch and twitch muscle units, and to a smaller extent also between the type S units of the sol gastrocnemius muscles (Fig. 7 Table 1) The former difference may simply reflect the ences in contraction time between fast and slow motor units. With the stimulation fre used the duration of each pulse train during the pre-tetanic stimulation would b sufficient to allow the fast twitch units to reach their maximal tension. The S units, o other hand, need longer tetanic stimulation in order to build up their peak tension (cf. S and Gersten 1967), which will here be close to tetanic fusion (Mosher *et al.* 1972), will therefore display greater t/p tension ratios. The observation that, despite their contraction times, the S units of the gastrocnemius muscle displayed a somewhat mean value for t/p tension ratio than did the SOL units ($p < 0.05$ Fig. 7 Table I), ma plained by the existence of potentiation in the former group of units only (Burke *et al.*

The difference between the S and FR types of units with respect to recovery fol the "pause test" is apparently not related to the state of fatigue only (Table I). Thus when the contractions of a type S unit decreased in amplitude during the "fatigue test was only negligible recovery after the pause. On the other hand, the FR units always sh an increase in tension after the pause, even in the cases where the fatigue index" ex 1 00. One explanation for this may be a greater ability of the FR units to recover from fatigue, thereby allowing a previously concealed potentiation to manifest itself. be significant, in this connection, that the intracellular enzymes of the FR muscle tend toward peripheral concentration, *i.e* close proximity to the extracellular supplies, whereas there is a more homogeneous intracellular distribution of enzymes type S muscle fibres (Olson and Swett 1966, Burke *et al* 1973 Hammarberg 1974). more important, however may be a relatively greater capacity of the FR fibres to rep their ATP supplies due to their larger contents of creatine phosphokinase (V R. Edg personal communication).

Acta physiol. scand. 1975, 95, 243–257

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The Postnatal Development of Some Twitch and Fatigue
erties of Single Motor Units in the Ankle Muscles of the Kitten

By

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Received 27 March 1975

Abstract

ARBERG, C. and J-O KELLERTH. *The postnatal development of some twitch and fatigue* *roperties of single motor units in the ankle muscles of the kitten.* Acta physiol. scand. 975, 95 243–257

ctions of single motor units in the gastrocnemius, soleus and pretibial flexor muscles were elicited ntracellular stimulation of the innervating motoneurones. Kittens being 1, 2, 6 and 10 weeks of ge sed. The aim was to establish the pattern of postnatal differentiation of the adult motor unit types et al. 1973, 1974, Hammarberg and Kellerth 1975 b). In the developing type S units of the soleus the mean value for contraction time showed transient decrease during the early postnatal period, e twitch half-relaxation time (HRT), the duration of motoneurone post-spike afterhyperpolarization) and the susceptibility to fatigue remained virtually unchanged during the age period studied. In ts of the developing fast twitch muscles the contraction time, HRT and susceptibility to fatigue were cantly different from those of the soleus units already at 1 week of age. At 6–10 weeks of age the ctile characteristics typical of the adult type FF and FR units were attained. The AHP duration ally decreased up to 10 weeks of age, and it was then considerably shorter than both at 1 week of ed in the adult stage.

ochemical observations suggest that fibres of the kitten hind limb muscles display ations in fibre size and enzyme content even before 1 week of age (Karpati and Engel 7, Nyström 1968 c, Hammarberg 1974). Although these muscles have been reported to ibit certain similar contractile properties during the first postnatal week (Banu 1922, ny-Brown 1929, Buller et al. 1960 a, Buller and Lewis 1965), functional differences ween them ha e also been described, particularly in their contractile responses to repe- ve stimulation (Buller and Lewis 1965, Nyström 1968 a, Hammarberg and Kellerth 75 a). This latter difference is of special interest, since the response patterns of single otor units to arious modes of repetitive stimulation ha been found to allow a functional ntification of different motor unit types in the adult cat (Olson and Swett 1971, Burke al. 1971, 1973, 1974, Hammarberg and Kellerth 1975 b). The extent to which histochemi- l and functional dissimilarities between immature kitten muscles may reflect an incipient fferentiation into arious motor unit types, however, could be determined only by studies the single unit level.

DEVANANDAN, M. S., R. M. ECCLES and R. A. WESTERMAN. Single motor units of mammalian muscle. *J. Physiol.* (Lond.) 1965. *178*. 359–367.

ECCLES, J. C., R. M. ECCLES and A. LUNDBERG. The action potentials of the alpha motoneurones supplying fast and slow muscles. *J. Physiol.* (Lond.) 1958. *142*. 275–291.

EULER, U. S. v. and R. L. SWANK. Tension changes during tetanus in mammalian and avian muscles. *Acta physiol. scand.* 1940. *1*. 201–219.

GORDON, G. and C. G. PHILLIPS. Slow and rapid components in a flexor muscle. *Quart. J. exp. Physiol.* 1953. *38*. 35–45.

GOSLOW, Jr. G. E., E. K. STAUFFER, W. C. NEMETH and D. G. STUART. Digit flexor muscles in the cat: Their action and motor units. *J. Morph.* 1972. *137*. 335–352.

HAMMARBERG, C., Histochemical staining patterns of muscle fibres in the gastrocnemius, soleus and anterior tibial muscles of the adult cat, as viewed in serial sections stained for lipids and succinic dehydrogenase. *Acta Neurol. Scandinav.* 1974. *50*. 272–284.

HAMMARBERG, C. and J-O. KELLERTH, The postnatal development of some twitch and fatigue properties of the ankle flexor and extensor muscles of the cat. *Acta physiol. scand.* 1975 a. *95*. 166–178.

HAMMARBERG, C. and J-O. KELLERTH. The postnatal development of some twitch and fatigue properties of single motor units in the ankle muscles of the kitten. *Acta physiol. scand.* 1975 b. *95*. 243–257.

HENNEMAN, E. and C. B. OLSON, Relations between structure and function in the design of skeletal muscles. *J. Neurophysiol.* 1965. *28*. 581–598.

KUNO, M., Excitability following antidromic activation in spinal motoneurones supplying red muscles. *J. Physiol.* (Lond.) 1959. *149*. 374–393.

LLOYD, D. P. C., After-currents, after-potentials, excitability and ventral root electrotonus in spinal motoneurons. *J. gen. Physiol.* 1951. *35*. 289–321.

MCPHEDRAN, A. M., R. B. WUERKER and E. HENNEMAN, Properties of motor units in a homogeneous red muscle (soleus) of the cat. *J. Neurophysiol.* 1965. *28*. 71–84.

MOSHER, C. G., R. L. GERLACH and D. G. STUART. Soleus and anterior tibial motor units of the cat. *Brain Res.* 1972. *44*. 1–11.

NYSTRÖM, B., Effect of direct tetanization on twitch tension in developing cat leg muscles. *Acta physiol. scand.* 1968 a. *74*. 319–330.

NYSTRÖM, B., Histochemistry of developing cat muscles. *Acta Neurol. Scandinav.* 1968 b. *44*. 405–439.

OGATA, T. A histochemical study of the red and white muscle fibers. *Acta Med. Okayama* 1958. *12*. 216–227.

OLSON, C. B. and C. P. SWETT Jr. A functional and histochemical characterization of motor units in a heterogeneous muscle (flexor digitorum longus) of the cat. *J. comp. Neurol.* 1966. *128*. 475–498.

OLSON, C. B. and C. P. SWETT Jr. Effect of prior activity on properties of different types of motor units. *J. Neurophysiol.* 1971. *34*. 1–16.

PROSKE, U. and P. M. E. WAITE, Properties of types of motor units in the medial gastrocnemius muscle of the cat. *Brain Res.* 1974. *67*. 89–101.

RENSHAW, B., Central effects of centripetal impulses in axons of spinal ventral roots. *J. Neurophysiol.* 1946. *9*. 191–204.

SEXTON, A. W. and J. W. GERSTEN, Isometric tension differences in fibers of red and white muscles. *Science* 1967. *157*. 199.

STANDAERT, F. G. The mechanisms of post-tetanic potentiation in cat soleus and gastrocnemius muscles. *J. gen. Physiol.* 1964. *47*. 987–1001.

STEPHENS, J. A. and D. G. STUART. The classification of motor units in cat medial gastrocnemius muscle. *J. Physiol.* (Lond.) 1974. *240*. 43 P.

WUERKER, R. B., A. M. MCPHEDRAN and E. HENNEMAN. Properties of motor units in a heterogeneous pale muscle (m. gastrocnemius) of the cat. *J. Neurophysiol.* 1965. *28*. 85–99.

...ed. It is seen that a considerable variation in duration of AHP was already present ...eek of age, the values ranging between 60 and 180 ms. However no significant differ... was found between motoneurones innervating the potentially fast twitch and slow ... muscles, the mean values for AHP duration being 122.3 ± 31.9 ms and 125.7 ± 21.4 ...espectively

...e AHP of the SOL motoneurones changed its mean duration very little as kitten age ...sed, although there were too few observations in the older age groups to allow any ...rm conclusions. In the adult animals, however the soleus AHP's were of significantly ...r duration (198.0 ± 30.0 ms) than in the youngest kittens ($p < 0.001$). The mean values ...HP duration of motoneurones innervating the potentially fast twitch muscles remained ...ally unchanged during the first 2 postnatal weeks (125.2 ± ...6.7 ms at 2 weeks of age). ...e older kittens, however a gradual and conspicuous decrease in duration of the AHP's ... place, the lowest values being encountered at around 10 weeks of age (61.2 ± 14.0 ms). ...e adult animals, however the mean duration of AHP of the fast twitch FF + FR units ... increased significantly (105.5 ± 34.3 ms, $p < 0.001$). Although the adult values for AHP ...ation seemed to be attained at a rather late postnatal stage for each motoneurone type, ... *difference* in mean value for AHP duration between the "tonic" and "phasic" moto...rones was rather well established by 10 weeks of age, the mean values here being 132.4 ± ...16 ms and 61.2 ± 14.0 ms ($p < 0.001$), respectively

...ch contraction time (time-to-peak)

...e changes in the speed of the muscle unit contractions with increasing age have been ...strated in Figs. 1 and 6. In the youngest kittens the values for contraction time varied ...ween 30 and 80 ms, and even at this age the SOL units were clearly situated on the slow ...tch side of the spectrum (Fig. 1). In fact, the mean values for contraction time of the ...L units were quite similar in the 1 week-old kittens (75.1 ± 7.6 ms) and adult cats (76.4 ± ...6 ms). At 2 weeks of age the contraction times had generally decreased (56.0 ± 15.6 ms, ... 0.001) This is consistent with the results of previous studies (Buller *et al.* 1960 a, Mann ...d Salafsky 1970, Hammarberg and Kellerth 1975 a) where the contraction time of the ...ole SOL muscle was found to decrease during the first 3 postnatal weeks, and to later ...crease towards adult values.

The majority of immature muscle units in the potentially fast twitch muscles gradually ...creased their contraction times with increasing age. There was small but significant ... 0.05) difference between the 1-week-old (44.8 ± 8.0 ms) and 2-week-old (36.6 ± 12.8 ms) ...ttens in this respect, and at 6 weeks of age substantial decrease had occurred (27.2 ± 12.3 ...ms, p 0.001). At 10 weeks of age the contraction times displayed a mean value of 21.8 ± 4.7 ...ms, which is similar to that of the fast twitch muscle units of adult cats (Hammarberg and ...ellerth 1975 b). In some units of these muscles the slow contractions persisted throughout ...e postnatal period. The fatigue properties of number of such slow twitch units were ...nvestigated at 6 and 10 weeks of age, and all of these units were found to exhibit a behaviour ...more or less identical with that of the adult type S units (Hammarberg and Kellerth 1975 b).

... Fig. 1 also shows the relationship between motoneurone AHP and twitch time-to-peak

The aims of the present investigation are to describe the pattern of postnatal diffe- tion of motor units into the various adult unit types (*cf* Burke *et al.* 1973, 1974, Ha- berg and Kellerth 1975 b) and to compare the behaviour of single units to that of the co- sponding whole muscles (Hammarberg and Kellerth 1975 a). The results will be rel- to subsequent ultrastructural studies of the postnatal changes in synaptic rela- nections of different types of motoneurones (Ronnevi and Conradi 1974, Hamma- Kellerth and Ronnevi in preparation)

Methods

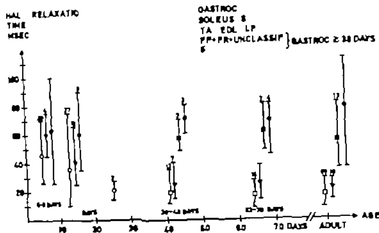
The general methods used in the present experiments have been described in detail in preceding (Hammarberg and Kellerth 1975 a, b). Briefly kittens on pentobarbitone anaesthesia (35-40 mg/kg ventilated artificially and held in a frame immobilizing the vertebral column and the right hind The medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (SOL), anterior tibial (TA), ex digitorum longus (EDL) and peroneus longus (LP) muscles were dissected free and their tendon arranged for separate isometric connection to a strain gauge myograph. The MG LG + SOL, and com peroneal nerves were prepared in the popl teal fossa and mounted intact on stimulating electrode lumbosacral laminectomy was performed and the dorsal roots were reflected medially to allow a be pproach to the ventral grey matter In the older kittens the dorsal roots L5-S2 on the right side wer but in animals less than 3 weeks of age the roots were kept i tact in order to permit orthodromic acti f the moto pools, since antidromic in asion of the motoneurones is often not possible at that (Kellerth *et al.* 1971). Exposed tissues were covered with mineral oil. Both body temperature and temperature of the oil pools were maintained by infrared light and monitored continuously Intramu temperature was checked at gular intervals. Temperature control was often difficult in the you kittens because of small oil volumes and small body sizes, and variations between 34 and 37 C ha accepted The measured value f contraction time of each muscle unit (= muscle fibres innervated b si gle mot neurone) was later adjusted t the corresponding alue at 38 C by using the correction $T_{10} = 1.55$ (Gordo and Phillips 1953 Buller *et al.* 1968). Intracellular micropipette electrodes wer to record from and to stimulate single motoneurones nnervating the dissected muscles. I the you age groups only a minor proportion of the motoneurones would allow an external e investigation be deteriorating. The EMG acti ty of 21 un ts, primarily n the 1 nd 2 week-old kittens, was made using fine monopolar steel wires hooked into the muscle surface over the dimpling produced by the c tracti g unit. Electrical and mechanical esponses of the motor units were recorded on an oscillo nd a DC penwriter (Devices M2) The testing procedure as well as the stimulation patterns used to eval the contractile properties of single muscle un ts, g. the "f tigue patte and "tetanus tests h been described in detail in p ecedi g reports (Hammarberg and Kellerth 1975 a, b).

Results

The present report is based upon a study of 147 motor units in 41 kittens. The selected a mals were approximately one week old (mean 7.2 days, S.D - 1 4 days, 17 animals, motor units), two weeks old (14.2 ± 1 1 days, 13 animals, 53 motor units), six weeks o (42.0 ± 3.8 days, 7 animals, 31 motor units) and ten weeks old (67 0 ± 2.4 days, 4 animals, motor units). The motor units investigated belonged to the SOL (26 units), MG (51 units) LG (43 units) TA (2 units), EDL (7 units) and LP (18 units) muscles.

Motoneurone post spike afterhyperpolarization (AHP)

The durations of motoneurone AHP's which were recorded at various postnatal ages have been plotted along the ordinates in the diagrams of Fig. 1 For comparison, the correspond- ing values of adult motoneurones (from Hammarberg and Kellerth 1975 b) have also bee



2. Graph showing the postnatal changes in twitch half-relaxation time for muscle units of different ... Each bar illustrates the range of observed values, while the superimposed symbols indicate the mean. The number of observations has been indicated above each bar.

...tch half-relaxation time (*HRT*)

... 2 shows the values for HRT of muscle units at various postnatal ages. In the animals ...re than 38 days old the units of Fig. 2 were classified into different types according to ... criteria discussed below and in a preceding report on adult motor units (Hammarberg ... Kellerth 1975 b). In the potentially fast twitch muscle units the mean value for HRT ...dually decreased with increasing age. In the gastrocnemius units this value changed from ... ± 13.0 ms at 1 week of age to 37.3 ± 16.2 ms ($p < 0.1$) at 2 weeks of age. At 6 weeks of ... the value (19.5 ± 7.8 ms) had not quite reached that of the 10-week-old kittens (16.2 ± ...ms) or adult animals (15.5 ± 5.2 ms, $p < 0.02$). The postnatal changes in HRT of the ...tor units followed a time course similar to that of the extensor units, although the mean ...lue was somewhat larger in all the age groups studied.

Only minor changes in HRT mean value occurred in the SOL units during the post...tal period, which observation agrees with previous results from whole SOL muscles ...ammarberg and Kellerth 1975 a). At 1 and 2 weeks of age the mean HRT values of the ...OL units were 62.1 ± 25.2 ms and 60.9 ± 19.6 ms, respectively which values do not differ ...gnificantly from that of the adults (77.3 ± 26.6 ms, p 0.1). There was a significant differ...nce in HRT between units of the SOL and gastrocnemius muscles even at 1 week of age ... 0.05). In the older kittens and adult cats the SOL units had a larger mean value for ...HRT than did the gastrocnemius units classified as being of the S type (53.8 ± 15.3 ms, ... 0.05 in the adults).

Fatigue test

Fig. 3, 4 and 5 show three different motor units which together illustrate the spectrum of ...motor unit behaviours encountered in the 1-week-old kittens. In *A* of each figure is seen

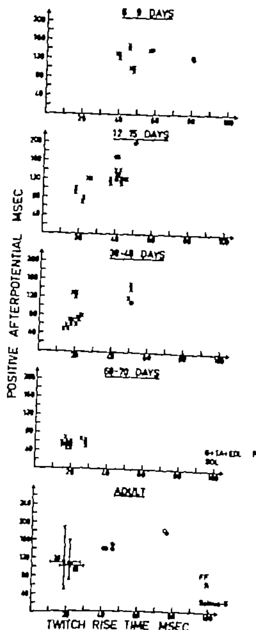


Fig. 1 Graph showing the relationship be[...] motoneurone post spike afterhyperpolarization ([...] nate) and muscle unit contraction time (ab[...] of single motor units at different postnatal ag[...]

at various postnatal ages. In the youngest kittens no relationship at all seemed to e[...] while at 6 weeks of age a clear change in the direction of the adult situation had occur[...] When the regression lines for the values of the three oldest age groups (38–48 days, 6[...] days and adult animals of Fig. 1) were calculated using the method of least squares, the [re]sulting slopes were 1.66, 1.89 and 1.61 respectively, showing that no dramatic change [in] this relationship occurred after 6 weeks of age. However, the corresponding y-interce[pts] were quite dissimilar in the three age groups (49 ms, 21 ms and 72 ms, respectively) and [re]flect in particular the general prolongation in motoneurone AHP which occurs after [the] 10th postnatal week (see above).

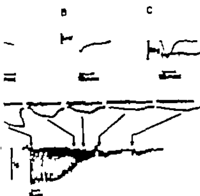


Fig. 4

A B C D

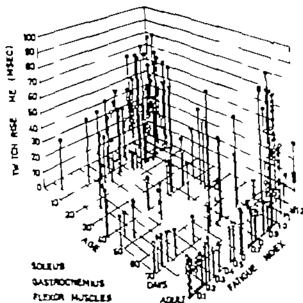
Fig. 5

MG motor unit of 6-day-old kitten. *A* Motoneurone action potential. *B*. Motoneurone AHP …tch contraction elicited by intracellular single-pulse stimulation of the motoneurone. *D* Continuous …ng of the contractile tension produced by the muscle unit during the "fatigue" "pause" and "tetanus" …(bottom), and *simultaneously at faster time base, the EMG records and tensions produced during* …the test sequence at the moments indicated by arrow (top).

SOL motor unit of an 8-day-old kitten. *A*. Motoneurone action potential. *B* Motoneurone AHP …itch contraction elicited by intracellular single-pulse stimulation of the motoneurone. *D*. Continuous …ding *of the contractile tension produced by the muscle unit during the "fatigue"* "pause and …nus tests.

…se test

…n 2 min of the "fatigue test had elapsed each unit was allowed to rest for 10 s, after …ch the iterative stimulation was resumed (see Fig. 3 4 and 5). Changes in contractile



…g. 6. Three-dimensional plot …owing the relationship between … contraction time (vertical …xis), "fatigue index (horizontal Z axis) and postnatal age …orizontal X-axis) for muscle …its of different muscles.

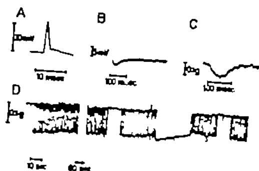


Fig. 3 MG motor unit of a 6-day-old kitten ... neurone action potential. B, Motoneurone ... Twitch contraction elicited by intracellular ... stimulation of the motoneurone. D, Continuous ... of the contractile tension produced by the ... during the fatigue "pause" and "tetanus" ...

the motoneurone action potential, in B the motoneurone AHP in C the twitch con... of the muscle unit and in D the contractile responses of the muscle unit to the "... "pause" and "tetanus tests" (Hammarberg and Kellerth 1975 a, b). Despite the ... the three immature units exhibited clear differences in some of their responses, part... in those illustrated in D of Fig. 3 4 and 5 For example, the fast twitch (contraction ... 32 ms) MG unit of Fig. 4 was extremely susceptible to fatigue (Fig. 4 D), while the ... twitch (contraction time ~89 ms) SOL unit of Fig. 5 was quite fatigue resistant (Fig. ... The unit of Fig. 4 however represents a rather extreme case of fatigue sensitivity at ... postnatally (cf Fig. 6), while a more "normal" behaviour at this age is exhibited b... MG unit shown in Fig. 3

The "fatigue indices" (Burke et al 1971 1973 Hammarberg and Kellerth 1975 a, b) ... on the whole rather large in the smallest kittens, indicating a well developed resista... fatigue in the majority of immature units (Fig. 6). Of the 69 units studied in kittens ... 1 and 2 weeks of age, 52 had fatigue indices exceeding 0 75 (Fig. 3 5 and 6). In the ... cat this value has been used as the lower limit for classifying the fatigue resistant FR ... unit types (Burke et al 1971 1973 Proske and Waite 1974 Hammarberg and Kell... 1975 b). However even in the smallest kittens some units were quite susceptible to fat... (Fig. 4 and 6) which indicates the possibility that the differentiation into adult motor ... types had already commenced It should also be pointed out that even in the 1 and 2 w... old kittens the SOL units were among the most fatigue resistant, with a mean value ... "fatigue index" of 1 00 ± 0 11 This value is larger than that of the gastrocnemius ... (0.73 ± 0.21 p < 0 001) at the same age. Compared with the gastrocnemius units the cor... sponding value for the flexor units of the youngest kittens was surprisingly large (0.9... 0 12, p < 0.001), but one should realize that in certain other respects also (see below, ... Hammarberg and Kellerth 1975 b) the flexor unit types may not be quite equivalent to ... extensor units. With increasing age a gradual differentiation took place among the ... of the fast twitch muscles. This development is reflected in the larger proportion of fatigue ... sensitive units ("fatigue index" < 0.25) found at 6 and 10 weeks of age (Fig. 6). The sl... twitch units of the SOL, on the other hand, showed only non-significant (p > 0.1) chang... in fatiguability with increasing age, the mean value for "fatigue index" being 0.97 ... at 6–10 weeks of age, and 0.94 ± 0.06 in the adult animals.

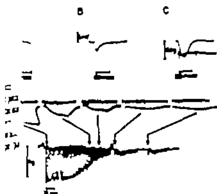


Fig. 4

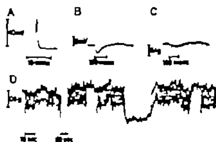


Fig. 5

MG motor unit of 6-day-old kitten. *A*. Motoneurone action potential. *B*. Motoneurone AHP. *C*. Twitch contraction elicited by intracellular single-pulse stimulation of the motoneurone. *D*. Continuous recording of the contractile tension produced by the muscle unit during the "fatigue", "pause" and "tetanus" tests (bottom), and simultaneously at a faster time base, the EMG records and tensions produced during the test sequence at the moments indicated by arrows (top).

SOL motor unit of an 8-day-old kitten. *A*. Motoneurone action potential. *B*. Motoneurone AHP. *C*. Twitch contraction elicited by intracellular single-pulse stimulation of the motoneurone. *D*. Continuous recording of the contractile tension produced by the muscle unit during the "fatigue", "pause" and tetanus tests.

the text

n 2 min of the "fatigue test" had elapsed each unit was allowed to rest for 10 s, after which the iterative stimulation was resumed (see Fig. 3, 4 and 5). Changes in contractile

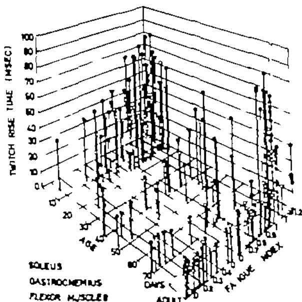


Fig. 6. Three-dimensional plot showing the relationship between contraction time (vertical axis), "fatigue index" (horizontal Z-axis) and postnatal age (horizontal X-axis) for muscle units of different muscles.

tension immediately following this pause were studied. In the adult anima usually allowed fast twitch units to increase their contractile tension, and thus to differentiate between FR and S units (Hammarberg and Kellerth 1975 b).

The "pause effect" measured in single units have been plotted along the al diagrams of Fig. 7 With one exception, the values ranged between 0–20 i age group (6–9 days), but one week later (12–15 days) they have started to spr abscissa, a tendency that is even more pronounced at 6–10 weeks of age. In l units at 38–70 days of age and in the adult stage have been omitted, since, due tensions usually produced by these units by the time of the "pause test (see Fi marberg and Kellerth 1975 b) considerable error was often introduced into the of the relative changes in contractile strength

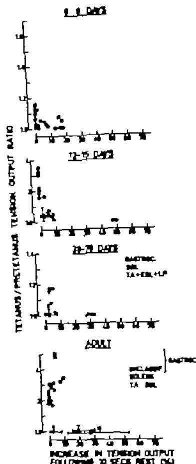
It is evident from Fig. 7 that the fatigue resistant type S units were grouped part of the spectrum at all ages, which means that they remained more or less by the "pause test" throughout the postnatal period. The fast twitch extensor u other hand, became more affected by the pause with increasing age. In the ad amount of recovery in contractile tension following the 10 s pause was not s ($p > 0.1$) related to the state of fatigue of the units. This is also illustrated in Fig. FR units ("fatigue index" > 0.75) and "unclassified" units ("fatigue index < 0.25 of the extensor muscles showed a considerable overlap in "pause effects (see also berg and Kellerth 1975 b).

The units of the flexor muscle responded to the pause test" in a way quite simi of the extensor type S units, *i.e.* the effects were small in all age groups studied (Fi was quite unexpected considering both the rather wide range of "fatigue indices by the flexor units (Fig. 6) and the large pause effect" obtained in whole flexo (Hammarberg and Kellerth 1975 a). However the flexor units of Fig. 7 probably co a skewed sample since as many as thirteen of the fifteen units in the 38–70-day-ol and adult cats were identified as FR or "unclassified' units with "fatigue indices between 0 62 and 0 97 It may be concluded from Fig. 7 though, that these units re to the "pause test" quite differently than did the corresponding motor unit typ extensor muscles. The large "pause effect found in the study of whole flexor (Hammarberg and Kellerth 1975 a) may possibly be attributed to FF units which represented in Fig. 7

"Tetanus test"

The "fatigue indices" in kittens around 1–2 weeks of age indicated that the muscle u the gastrocnemius were generally more sensitive to fatigue than those of the SOL and muscles (see above). A similar tendency was also found with the tetanus test, wh gastrocnemius units of the 1 and 2 week-old kittens, on the whole, exhibited a relative decrease in tension (39.6 ± 21.7 %, $n = 15$) during the course of sustained stimulation, than did the units of the pretibial flexors (24.3 ± 20.2 %, $n = 13$ $p < 0.1$). ever there was no significant difference in the decrease in tetanic tension between the gastrocnemius and SOL (29.4 ± 14.2 %, $n = 12$, $p > 0.1$) muscles of this age.

At 6–10 weeks of age the tension of the SOL units decreased by only 4.5 ± 2.1% (



...7. Graphs showing the postnatal changes in the relation between tetanus/pretetanus tension ratio (ordinate) and ...se effect" (abscissa) of muscle units of different muscles.

...n start to end of the tetanus, while the flexor units appeared somewhat more sensitiv... the tetanus than previously and had a corresponding value of 35.1 ± 22.4% (n = 9, p > 0.1... gastrocnemius units of the same age had tetanus responses which were related to the... these indices — that is, all the units corresponding to the FF type (n = 7) were complete... ...austed by the tetanus (Hammarberg and Kellerth 1975 b), and the FR units showed... ...nic tension decrease (26.9 ± 27.3%, n = 8) which was smaller than that of the "unclas... ...d" units (88.3 ± 21.7, n = 7, p < 0.001).

In the adult animals the values for tetanic tension decrease were 0.4 ± 1.2% (n = 8) f... ...the SOL units and 89.7 ± 9.9% (n = 10) for the gastrocnemius "unclassified" units (Hamm... ...rg and Kellerth 1975 b). In contrast to the situation in the kittens, both the FR and ...nits of the adult gastrocnemius showed an average *increase* in tension during the teta... ...f 24.4 ± 29.1% (n = 13) and 7.4 ± 10.2% (n = 7), respectively, due probably to potentiat... ...ffects induced by the tetanic stimulation (Standaert 1964, Nyström 1968 a, Olson ... ...wett 1971, Hammarberg and Kellerth 1975 b). The FR units of the flexor muscles, on

other hand, decreased in tetanic tension in all the cases studied (22.0±15(Hammarberg and Kellerth 1975 b).

In adult animals, as seen in Fig. 7 the tetanus/pretetanus (t/p) tension : "pause test" clearly separated the gastrocnemius FR and S types of units (and Kellerth 1975 b). In the 1 week-old kittens such a separation was not poss only one week later a clear tendency for arrangement according to the adult p seen (Fig. 7). In the older kittens, 6 and 10 weeks of age, the responses were qu those of mature muscle units. The gastrocnemius S units in the adult cats usua somewhat larger t/p tension ratios (1.35 ± 0.12) than did the SOL units (1.26±0. due possibly to the existence of potentiation in the former unit type only (Burk 1974). A similar difference can also be seen in the oldest kittens (Fig. 7) whe sponding ratios for gastrocnemius and SOL type S units were 1.3? ± 0.04 an ($p < 0.01$) respectively. The behaviour of the flexor units was quite different f the extensor units neither the "pause test" nor the "tetanus test" significantly tension output of the flexor units, which, often were also surprisingly resistant t the older kittens and adult cats (see above)

EMG activity during fatigue

The electromyographic activity of 21 units of the triceps surae muscle was rec majority of these units were from kittens belonging to the two youngest age grou The purpose of the EMG recordings was to ascertain whether or not the fatigue by single units was accompanied by a change in EMG response which would ind failure of factors other than the contractile mechanism might have been respons decrease in contractile tension. In all units where fatigue was present there was exception, a concomitant decrease in amplitude of the EMG potentials. The ex unit exhibited an increase in EMG amplitude during the fatigue. Generally the in contractile tension of a muscle unit was larger relatively ($p < 0.02$) than the c EMG amplitude (see *e.g.* Fig. 3) In no case was a "drop out" of spikes observ trains of EMG potentials, which indicated that the propagation of nervous impu adequate down to at least the branching point of the axon terminals.

Discussion

Motoneurone AHP

The AHP's of motoneurones innervating potentially fast twitch muscle fibres were to gradually decrease in duration with increasing age. This decrease may to some be attributed to postnatal changes in the permeability properties of the moton membrane, such as *e.g.* an increased efficiency of the ionic pumps. Similarly moton resting membrane potentials and action potentials have been found to increase in mag with age and to display adult values around 6 weeks postnatally (Kellerth *et al* However the apparent absence of postnatal changes in soleus AHP's is more diffic reconcile with permeability change, unless one assumes differences in the AHP gene mechanisms and/or in the state of maturity at birth between the two groups of motoneu

general increase in AHP duration which occurred in all motoneurones after 10 weeks was quite conspicuous, but one can only speculate about the mechanism underlying this age more or less mature conditions would be expected with respect to moto-n membrane properties (Kellerth et al. 1971), extracellular concentration of electro-Thelm 1973) and recurrent inhibitory effects (Mellström 1971). However although an cell body volume of lumbar motoneurones increases relatively little after 5–6 of age (Mellstrom and Skoglund 1969), the branching of their dendritic trees has ported to continue until 4–5 months of age (Scheibel and Scheibel 1970). Such an in distal dendritic membrane area may account for the prolongation of moto- AHP's in the older kittens.

contractions

natal changes in twitch contraction time and HRT were generally similar to those ed in a previous study on whole muscles (Hammarberg and Kellerth 1975 a). In ad- the mean alues for contraction time corresponded closely to those observed in of the SOL and flexor digitorum longus muscles at 2 and 6 weeks of age by Bagust 1974). The values for twitch speed and HRT were quite scattered even in the smallest s, possibly reflecting histochemical variations among muscle fibres at these ages (Ham- rg 1974), but the wide range of values typical of the adult animal was not established around 6 weeks of age, at which time most fast units displayed adult twitch character (cf Bagust et al. 1974). At this same age the difference in twitch duration between type FR and type S units was also close to that of the adult units (see Fig. 1 and 2). Con- ng the fact that contraction characteristics are at least partly dependent upon the lic type of neurone innervating the muscle fibres (Buller et al. 1960 a, b) one should that polyneuronal innervation (Bagust et al. 1973) as well as formation of new axonal cts (Bagust et al 1974) have been reported to occur in the kitten hind limb muscles ntil 6 weeks of age. Only around this age, therefore, would all muscle fibres be exposed constant and fully developed neuronal influence which would allow them to achieve final adult characteristics

ue properties

At 1 2 weeks of age considerable ariations were encountered in the responses of or units of the different muscles to the "fatigue "pause" and "tetanus tests This be explained by the fact that differentiation lot arious motor unit types had already menced, although response patterns entirely typical of the adult FF FR and S unit s (Hammarberg and Kellerth 1975 b) were not in the majority until 6–10 weeks of age. relatively large proportion of "unclassified units in evidence even at this age, however gests that maturation was not yet complete. The SOL units were generally found to be ong the least fatiguable even at 1 2 weeks of age, but the presence of potentiation in the L muscles of the smallest kittens (Nystrom 1968) ha e may concealed a "true" muscular gue (cf Hammarberg and Kellerth 1975 a).

With the stimulation frequencies used here, post-tetanic potentiation of muscle con- ctions in both kittens and adult cats (Nyström 1968 a) as well as fatigue of mature muscle

other hand, decreased in tetanic tension in all the cases studied (22.0±15 ...
Hammarberg and Kellerth 1975 b)

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lo developmental changes, or alternatively that most developmental changes occur lly With respect to muscle fibre diameters the S type units appear more mature at an the potentially fast twitch units (Engel and Karpati 1968, Hammarberg 1974).

l considerations

wborn kitten lacks the ability to stand and walk, but develops these functions during t three postnatal weeks, being able even to run after 5–6 weeks. The postnatal develop- of the postural and locomotor mechanisms responsible for these functions involves a of structural and functional changes in both the central and peripheral parts of the system (*cf* Skoglund 1960 a–c, Berthold 1968, Nystrom 1968 a, b, c, Conradi 1969 rom and Skoglund 1969 Mellstrom 1971).

present study found that the muscle units displayed adult functional characteristics und 6 weeks of age. The pattern of changes in histochemical appearance of the muscles a similar time course (Hammarberg 1974). This particular age has also been reported her authors to be a transition point between immature and adult properties in the system, *i.e.* after 6 weeks of age polyneuronal innervation of muscle fibres and tion of new axonal contacts does not occur (Bagust *et al.* 1973 1974), motoneurone potentials and resting membrane potentials show adult magnitudes (Kellerth *et al.* differences in morphological appearance of the motor nerve terminals of the gastro- us and soleus muscle fibres is evident (Nystrom 1968 b) and there is only a relatively additional increase in motoneurone cell body volume (Mellstrom and Skoglund).

certain other respects maturity of motor function may occur earlier than 6 weeks. The ctory period of immature fibres is about twice that of mature fibres (Hursh 1939), impulse frequencies can be mediated in a mature way in developing fibres with a 6 μm neter (Ekholm 1967), which in the ventral root fibres corresponds to an age of about eks (Skoglund and Romero 1965). At this age the ventral root fibres also display adult phological characteristics in the node-paranode region (Berthold 1968).

When considering the role of neurotrophic influences in the differentiation of motor ction (Buller *et al.* 1960 a, b), one should also pay attention to the elimination and re tribution of synaptic contacts which occur on lumbar motoneurones before 3 weeks of (Conradi 1968, Ronnevi and Conradi 1974 Conradi and Ronnevi 1975). The possible relation between such changes in synaptic reflex connections and the differentiation various motor unit types will be considered in subsequent physiological and ultrastruc al studies (Hammarberg in preparation, Hammarberg, Kellerth and Ronnevi, in prepa- ion).

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References

GUST J., D M. LEWIS and R. A. WESTERMAN, Polyneural innervation of kitten skeletal muscle. *J Physiol* (Lond.) 1973, 229 241–255.

GUST J. D M. LEWIS and R. A. WESTERMAN, The properties of motor units in fast and slow twitch muscle during post-natal development in the kitten. *J Physiol.* (Lond.) 1974, 237 75–90.

units (Burke *et al* 1973) appear to be events entirely restricted to the muscle fibr fatigue exhibited by muscle units of the 1 and 2 week-old kittens, however, was accompanied by a decreased amplitude of the EMG potentials of the units. For the relative depression in contractile tension was generally much greater than the EMG responses, a linear relationship between these two factors cannot be taken for (Lüttgau 1965) and any direct comparison of the fatiguability of immature and adult units is therefore hazardous. There is some support, however for the view that the *differences* in fatigue properties between units of the potentially fast and slow m the youngest kittens may not depend on differences in presynaptic mechanisms or muscular transmission, but rather on postsynaptic differences (see Discussion of H berg and Kellerth 1975 a)

The responses of single units to repetitive stimulation were generally consist those of the corresponding whole muscles (Hammarberg and Kellerth 1975 a). ception, however was found in kittens around 1 and 2 weeks of age, where single not exhibit the same large decrease in tension during the "tetanus test as did th muscles. This difference between the responses of single units and whole mus possibly be accounted for by ischemia arising secondarily to an occlusion of th supply and/or by extracellular accumulation of metabolites or potassium, during tetanic contraction of the whole muscles. Whatever the explanation for the above tion may be, it appears that the immature muscles, and particularly the potent twitch ones, are more sensitive to such influence than the adult muscles.

Differentiation of motor unit types

Statistically significant differences in twitch contraction time, twitch half-relaxa and susceptibility to fatigue between muscle units of potentially fast and slow twitch were observed at even 1 week of age. This indicates that differentiation into vario unit types starts at a very early age, possibly even at a prenatal stage (cf Ridge 1 though the contractile response patterns typical of the adult FF and FR motor u (Hammarberg and Kellerth 1975 b) are not fully developed until 6–10 weeks of ag

With the exception of contraction time, which showed a transient decrease du early postnatal period, the functional parameters investigated in the SOL units only insignificant changes from 1 week of age to the adult stage. This is combi findings in previous studies on whole SOL muscles (Hammarberg and Kellerth but may seem unexpected in view of the heterogeneity of SOL fibres in the younge revealed by histochemical stains for ATPase and lipids (Karpati and Engel 1967 1968 c, Hammarberg 1974) Postnatal variation in soleus ATPase activity and its disappearance at 7 weeks of age (Nyström 1968 c) may well account for the simu changes in contraction time, since a direct correlation between these two paramete to exist (Bárány 1967). Postnatal changes in lipid content (Hammarberg 1974) c however have necessarily been reflected in the types of functional tests used here.

The absence of obvious postnatal changes in functional properties of the type contrasts markedly to the pattern of differentiation of the fast twitch units. This m cate that the S units belong to a more primordial unit type which does not unde

R., Postnatal development of motor nerve terminals in "slow-red" and "fast white" cat muscles. *Neurol. Scandinav.* 1968 b. *44.* 363–383.

R., Histochemistry of developing cat muscles. *Acta Neurol. Scandinav.* 1968 c. *44.* 405–439

C. B and C. P SWETT J Effect of prior activity on properties of different types of motor units. *physiol.* 1971. *34.* 1–16.

U and P M. E. WALTZ, Properties of types of motor units in the medial gastrocnemius muscle cat. *Brain Res.* 1974. *67* 89–101

R. M. A. P The differentiation of conduction velocities of slow twitch and fast twitch muscle innervations in kittens and cats. *Quart J exp. Physiol.* 1967 *52.* 293–304.

L-O. and E. CONRADI, Ultrastructural evidence for spontaneous elimination of synaptic terminals spinal motoneurons in the kitten. *Brain Res.* 1974. *80.* 335–339.

M. E. and A. B. SCHEIBEL, Developmental relationship between spinal motoneuron dendrite and patterned activity in the hind limb of cats. *Exp. Neurol.* 1970. *29* 328–335.

S On the postnatal development of postural mechanisms as revealed by electromyography myography in decerebrate cats. *Acta physiol. scand.* 1960 a. *49* 299–317

S., The spinal transmission of proprioceptive reflexes and the postnatal development of conduction velocity in different hind limb nerves in the kitten. *Acta physiol. scand.* 1960 b. *49* 318–329

S., The activity of muscle receptors in the kitten. *Acta physiol. scand.* 1960 c. *50.* 203–221

S., Central connections and functions of muscle nerves in the kitten. *Acta physiol. scand.* 1960 d. 222–237

S., The reactions to tetanic stimulation of the two-neuron arc in the kitten. *Acta physiol. scand.* 1960 e. *50.* 238–253.

S. and C. ROMERO, Postnatal growth of spinal nerves and roots. A morphological study in the with physiological correlations. *Acta physiol. scand.* 1965. *66.* *Suppl.* 260.

P G., The mechanisms of post-tetanic potentiation in cat soleus and gastrocnemius muscles. *J. gen. Physiol.* 1964 *47* 987–1001.

BANU, G Recherches physiologiques sur le développement neuromusculaire. Paris 1922.
BÁRÁNY M ATPase activity of myosin correlated with speed of muscle shortening. J. gen. Physiol. 50, 197 18
BERTHOLD, C H., Ultrastructural and light microscopical features of postnatally developing of feline peripheral, myelinated nerve fibres. 1968. Brogenhardt tryckeri AB. Lidingö.
BULLER, A. J and D M LEWIS, The rate of tension development in isometric tetanic contr— mammalian fast and slow skeletal muscle. J Physiol. (Lond.) 1965. 176. 337–354.
BULLER, A. J., J C. ECCLES and R M ECCLES, Differentiation of fast and slow muscles in the limb. J Physiol. (Lond.) 1960 a. 150. 399–416.
BULLER, A J., J C. ECCLES and R. M ECCLES, Interactions between motoneurones and muscles of the characteristic speeds of their responses. J Physiol. (Lond.) 1960 b. 150. 417–439.
BULLER, A. J K. W RANATUNGA and J M SMITH, The influence of temperature on the characteristics of mammalian fast and slow twitch skeletal muscles. J Physiol. (Lond.) 1968. 19
BURKE, R. E., D N LEVINE, F E. ZAJAC, III P TSAIRIS and W K. ENGEL, Mammalian motor Physiological-histochemical correlation in the three types in cat gastrocnemius. Science 1971. 709–712.
BURKE, R E. D N LEVINE, P TSAIRIS and F E. ZAJAC, III Physiological types and histochemical in motor units of the cat gastrocnemius. J Physiol. (Lond.) 1973. 234 723–748.
BURKE, R. E., D N LEVINE, M. SALCMAN and P TSAIRIS, Motor units in cat soleus muscle: Physiol. histochemical and morphological characteristics. J Physiol. (Lond.) 1974. 238. 503–514.
CONRADI, S., On motoneuron synaptology in cats and kittens An electron microscopic study of the co- and location of neuronal and glial elements on cat lumbosacral motoneurons in the normal ca after dorsal root section. 1969 Tryckeribolaget Ivar Häggstrom. Stockholm.
CONRADI, S. and L-O RONNEVI, Spontaneous elimination of synapses on cat spinal motoneurons birth do half of the ynapses on the cell bodies disappear? Brain Res. 1975 92. 505–518.
CULLHEIM, S., A study of plasma and CSF potassium relationship during postnatal development cat. Act physiol. scand. 1973. 89. 594–599
DENNY BROWN, D E., The histological features of striped muscle in relation to its functional Proc roy soc B 1929 104 371–411
EKHOLM, J Postnat l changes in cutaneous reflexes and i the discharge pattern of cutaneous and sense organs. A morphological and physiological study in the cat Acta physiol. scand. 1967 Suppl
ENGEL, W K. and G KARPATI, Impaired skeletal muscle maturation following neonatal neurect Develop Biol. 1968. 17 713–723.
GORDON G and C G PHILLIPS, Slow and rapid components i a flexor muscle. Quart J. exp. Ph 1953 38 35–45
HAMMARBERG, C., The histochemical appearance of developing muscle f bres in the gastrocnemius and anterior tibial muscles of the kitten, as iewed n serial sections stained for lipids and dehydrogenase. A I Neurol Scandinav. 1974 50 485–301
HAMMARBERG, C. and J O KELLERTH, The postnatal development of some twitch and fatigue prop of the ankle flexor and extensor muscles of the cat. Act physiol. scand. 1975 a. 95. 166–178.
HAMMARBERG, C. and J-O KELLER H, Studies of some tw tch and fatigue properties of different unit types in the ankle muscles of the adult cat Act physiol scand. 1975 b. 95. 231 42.
HURSH, J B The properties of growing nerv f bers. Amer J Physiol. 1939 127 140–153.
KARPATI, G and W K. ENGEL, Neuronal tr phic function. Arch Neurol. 1967 17 54– 545.
KELLERTH, J O A. MELLSTRÖM and S. SKOGLUND, Postnatal excitabil ty changes of kitten motone Acta physiol. scand. 1971 83 31–41
LÜTTG u, H C., The effect of metabolic nh bitors on the fatigue of the action potential in single fibres. J Physiol. (Lond.) 1965 178 45–67
MANN W S. and B. SALAFSKY Enzymic and physiological studies n normal nd disused develop and slow cat muscles. J Phy iol (Lond.) 1970 208 33 47
MELLSTRÖM, A., Recurrent and tidromic effects on the monosynaptic reflex during postnatal d ment in the cat. A ta physiol. cand. 1971 82 490–499
MELLSTRÖM, A and S. SKOGLUND, Quant tativ morphological cha ges in some spinal cord se d ring postnatal development A tudy the cat A ta ph siol scand. 1969 Suppl. 331
NYSTRÖM, B. Mechanical and electrical responses to singl shocks in developing cat leg muscles (af tetanization. Acta physiol. scand 1968 74 707 225.

[illegible] I. Secretory responses to gastrin in Pavlov pouch dogs with and without perfusion of the duodenal bulb with 0.1 N HCl.

	Number of expts.	Control hours		Mean secretory rate expressed as percentage of the secretory level during the control hour. Half hour periods following the control hour			
		Range of secretory levels, mmol/15 min	Relative S.E. of mean of four 15-min periods (per cent)	1	2	3	4
[illegible]	3	0.45–0.51	9	30	10	39	96
[illegible]	3	0.19–0.67	11	18	8	21	70
	3	0.29–0.47	11	67	15	23	101
		Mean ± S.E. of mean		41±7	11±7	28±6	89±11

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...common methods of analysis of variance were used (Snedecor 1967) for the statistical evaluation of the ...ts.

Results

...bar perfusion with acid profoundly inhibited acid responses to exogenous gastrin in all ...s (Table I). When similar gastric secretory rates were induced by Urecholine in the same ...s, bulbar acidification produced some reduction of the acid output in one dog (A). ...wever the inhibitory pattern was different from that observed in experiments with ...trin where inhibition of acid secretion consistently became most pronounced during the ...ond half-hour of bulbar acidification. In dog B acidification of the bulb did not signifi...ntly influence the acid output from the gastric pouches, whereas in dog C an increase ...the acid output was observed following perfusion of the bulbar pouch with acid. These ...ults are presented in Table II.

Discussion

...a series of studies in dogs attempts have been made to reveal the characteristics of the ...echanism by which acid in the duodenal bulb inhibits acid secretion. From these results ...appears that the bulbar mechanism is very effective in inhibiting acid responses to exo...nous gastrin (Andersson, Nilsson and Uvnäs 1967, Andersson and Nilsson 1969, Nilsson ...d Rune 1971, Nilsson 1974, Nilsson 1975 c) or to stimuli causing release of gastrin such ...s test meals (Andersson and Uvnäs 1961, Andersson and Sjödin 1972, Nilsson 1975 b), ...ham feeding (Nilsson 1969, Andersson and Sjödin 1972, Nilsson 1975 a) or insulin hypo...lycemia (Nilsson 1969, Andersson and Sjödin 1972). Also in the present study bulbar ...cidification profoundly inhibited acid responses induced by exogenous gastrin, which ...hows that the bulbar mechanism was very efficient in inhibiting acid secretion in each

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Effect of Bulbar Acidification on Gastric Acid Responses to Urecholine in Pavlov Pouch Dogs

By

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Received 27 March 1975

Abstract

NILSSON, G *Effect of bulbar acidification on gastric acid responses to Urecholine in Pavlov pouch dogs.* Acta physiol scand 1975 95 258–262.

Dogs were provided with mucosal septal pouches of the fundic stomach and of the duodenal bulb. Acid secretion was stimulated by intra enous infusion of submaximal doses of porcine gastrin or the choline ester Urecholine. Acid perfusion of bulbar pouches profoundly inhibited acid responses to gastrin. Bulbar acidification produced little or no reduction in acid secretion induced by Urecholine. Small doses of Urecholine do not release gastrin in th dog but may induce acid secretion, mainly by direct cholinergic stimulation of the HCl glands. The fact that Urecholine-induced acid secretion was not inhibited in the present experiments is consistent with the hypothesis that the bulbar mechanism does not inhibit cholinergic stimulation acting on the parietal cells.

Recent experiments (Sjödin and Nilsson 1974) have shown that intravenous infusion of moderate doses of the stable choline ester Urecholine evokes acid secretion without changing plasma levels of gastrin This observation may mean that Urecholine activates the glands mainly by direct cholinergic stimulation The present study was undertaken to determine whether bulbar acidification influences gastric acid responses to cholinergic stimulation induced by Urecholine.

Methods

Double septal pouches (Pa lov type) of the fundic stomach and of the duodenal bulb (Andersson and 1961) were constructed in 3 mongrel dogs weigh ng 15–17 kg. Between operations and before starting experiments the dogs were allowed a period of 3 weeks for recovery

Experiments were started in the morning after the dogs had been fasted for 18–20 h. Gastric juice was collected from Pa lov pouches in 15 min portions. The volume was measured and the acidity was determined by titration with 0.01 M Na OH, using phenolphthalein as indicator Before an experimental series was started gastric acid responses to graded doses of Urechol ne were determined in each dog. From these experiments a dose (15 µg/kg/h) of Urecholine was selected which evoked acid responses from the pouches corresponding 30–40 of the maximal secretion to Urecholine. In each experiment Urecholine was given in a 0.9% solution of NaCl which was infused by a calibrated peristaltic pump (Harvard Apparatus Co., Dover Mass.) at rate of 20 ml per hour When, after 2 1/2 h infusion, a st

[Table] I. Secretory responses to gastrin in Pavlov pouch dogs with and without perfusion of the duodenal bulb with 0.1 N HCl.

Number of expts.	Control hours		Mean secretory rate expressed as percentage of the secretory level during the control hour. Half hour periods following the control hour			
	Range of secretory levels, meq/15 min	Relative S.E. of mean of four 15-min periods (per cent)	1	2	3	4
3	0.45–0.31	9	38	10	39	96
3	0.19–0.67	13	18	8	21	70
3	0.23–0.47	11	67	15	23	101
	Mean ± S.E. of mean		41 ± 7	11 ± 7	28 ± 6	89 ± 11

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TABLE II Secretory responses to Urecholine in Pavlov pouch dogs with and without [illegible] duodenal bulb with 0.1 N HCl.

Dog	Number of expts.	Control hours		Mean secretory rate expressed as percentage of the secretory level during the control hour. Half hour periods following the control hour			
		Range of secretory levels, meq/15 min	Relative S.E. of mean of four 15-min periods (per cent)	1	2	3	4
A	4	0.37–0.55	5	*65*	*67*	*96*	[illegible]
B	4	0.30–0.60	8	*112*	*108*	*112*	[illegible]
C	4	0.20–0.38	7	*126*	*154*	*166*	[illegible]
		Mean ± S.E. of mean		*101±14*	*110±12*	*125±13*	[illegible]

dog. However little or no inhibition occurred in experiments in which acid secretion [illegible] induced by Urecholine.

In order to understand the significance of the present results the nervous and hormonal factors involved in the activation of the HCl glands following cholinergic stimulation [illegible] be considered. It is well documented that the HCl glands have to be stimulated both vagally and by gastrin to secrete optimally (Uvnäs 1942, Olbe 1964, Sjödin 197?). Thus, resection of the gastric antrum and the duodenal bulb almost completely abolish acid responses to small doses of insulin (Olbe 1964) or to short periods of sham feeding (Olbe 1964, Sjödin 1972). Under certain conditions of vagal stimulation however it seems as if gastrin plays a minor role in the activation of the HCl glands. For example large doses of insulin (Pevsner and Grossman 1955 Olbe 1964) or prolonged sham feeding (Sjödin 1972) produce [illegible] secretion even after resection of gastrin-releasing tissues. Previous studies show that bulbar acidification abolishes acid responses to small doses of insulin (Nilsson 1969 a) and short periods of sham feeding (Nilsson 1969 b) whereas acid responses to large doses of insulin (Nilsson 1969 a) or to prolonged sham feeding (Nilsson 1969 b) are only partially inhibited. It was suggested (Nilsson 1969 c) from those observations (Pevsner and Grossman 1955 Olbe 1964 Nilsson 1969 a and b) that the bulbar inhibitory mechanism does not inhibit acid responses to stimulation under conditions in which the parietal cells can be subjected to a strong direct cholinergic excitation

The stable choline ester Urecholine, in the doses used in the present study evokes considerable acid responses in Pavlov pouch dogs without significantly raising the plasma gastrin concentration (Sjödin and Nilsson 1974) The mechanism by which Urecholine induces acid secretion is not fully understood Possibly basal levels of gastrin suffice to act synergistically with Urecholine stimulation on the parietal cells. Alternatively Urecholine may act essentially directly on the HCl glands, independent of gastrin or Urecholine may act through some other as yet unidentified mechanism.

Since the bulbar mechanism is very efficient in inhibiting acid responses to gastrin, and since gastrin levels are not elevated by stimulation with moderate doses of Urecholine one would expect that Urecholine-induced acid secretion would be profoundly inhibited if gastrin played a significant role in the activation of the HCl glands during Urecholine stimulation. However if Urecholine mainly induces acid secretion by a direct cholinergic

tion of the parietal cells, the present results support the previously proposed hypo- (Nilsson 1969 c) that the bulbar mechanism does not interfere with direct cholinergic lation of the acid secreting glands.

…e possible physiological role of the mechanism in the duodenal bulb inhibiting acid …tion may be to contribute to the suppression of postprandial acid secretion and to …e acid secretion that may occur from the empty stomach *during* interdigestive periods. presence of such a mechanism has been indicated not only in dogs but also in man …ston and Duthie 1965 Wormsley 1970) and in the rat (Lundberg and Andersson 1974). …ew of the present and previous (Nilsson 1969 a and b) results suggesting that the …ar mechanism does not influence direct cholinergic stimulation of the parietal cells, …ay be pertinent to discuss the role the bulbar mechanism may play in inhibiting acid …tion in patients with duodenal ulcer disease. It has been suggested that vagal hyper …ulation causes gastric hypersecretion in duodenal ulcer disease (Dragstedt 1942). If such …ulation of the parietal cells predominates in this disease, inhibitory influences arising …acidification of the bulbar mucosa may be less effective in reducing acid secretion in … duodenal ulcer patient than in normal man.

…is study was supported by research grants from Statens Medicinska Forskningsråd (3521) and from …olinska Institutet. The author is grateful to Miss Gunilla Tholin, Miss Elisabeth Tröjo and Miss Sara …lenowska for technical and secretarial assistance

References

…ERSSON, S. and G. NILSSON, pH-dependence of the mechanism in the duodenal bulb inhibiting gastric …id responses to exogenous gastrin. *Acta physiol. scand.* 1969 76, 182–190.

…DERSSON, S. and L. ROLNY, Inhibition of gastric secretion by acidification of the duodenal bulb before …nd after transection of the pylorus. *Scand. J. Gastroent.* 1972, 7 151–156.

…DERSSON, S. and B. UVNÄS, Inhibition of postprandial gastric secretion in Pavlov pouches by instillation …f hydrochloric acid into the duodenal bulb. *Gastroenterology* 1961 41 486–490.

…NDERSSON, S. G. NILSSON and B. UVNÄS, Effect of acid in proximal and distal duodenal pouches on …gastric secretory responses to gastrin and histamine. *Acta physiol. scand.* 1967 71 368–378.

…AGSTEDT L. R. Pathogenesis of gastroduodenal ulcer. *Arch. Surg.* 1942, 44, 438–450.

…NSTON, D and H L. DUTHIE, Inhibition of gastric secretion in the human stomach. *Lancet* 1965, 2, 1032–1036.

…NDBERG, B and S. ANDERSSON, The role of the upper duodenum in controlling gastric acid secretion in the rat. *Scand. J. Gastroent.* 1974, 9 623–627

…LSSON, G. Effect of acid in the duodenal bulb on gastric secretory responses to insulin hypoglycemia. *Acta physiol. scand.* 1969 75 476–483.

…LSSON, G. Effect of acid in the duodenal bulb on gastric secretory responses to sham feeding. *Acta physiol. scand.* 1969 b 77 308–315.

…LSSON, G. *Studies of the mechanism by which acid in the duodenal bulb inhibits gastric acid secretion.* Dissertation. Stockholm. 1969 a.

…LSSON, G. Effect of acidification of totally denervated pouches of the duodenal bulb on gastric acid secretion in dog. *Acta physiol. scand.* 1974, 92, 560–571.

…LSSON, G. Increased plasma gastrin levels in connection with inhibition of gastric acid responses to sham feeding following bulbar perfusion with acid in dogs. *Scand. J. Gastroent.* 1975 a. 10, 273–278.

…LSSON, G. Effect of acid in the duodenal bulb on acid and pepsin responses to test meal in dog. *Scand. J. Gastroent.* 1975 b. 10, 279–283.

…LSSON, G. Effect of bulbar perfusion with acid, hypertonic solutions and acetylcholine on gastric acid secretion in dog. *Scand. J. Gastroent.* 1975 c. 10 284–288.

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In order to understand the significance of the present results the nervous and [hormonal] factors involved in the activation of the HCl glands following cholinergic stimula[tion must] be considered. It is well documented that the HCl glands have to be stimulated bot[h by nerves] and by gastrin to secrete optimally (Uvnäs 1942, Olbe 1964 Sjödin 1972). Thus, [resection] of the gastric antrum and the duodenal bulb almost completely abolish acid resp[onses to] small doses of insulin (Olbe 1964) or to short periods of sham feeding (Olbe 196[4] 1972) Under certain conditions of vagal stimulation, however it seems as if gast[rin plays] a minor role in the activation of the HCl glands. For example large doses of insul[in (Pev-]ner and Grossman 1955 Olbe 1964) or prolonged sham feeding (Sjödin 1972) prod[uce acid] secretion even after resection of gastrin-releasing tissues. Previous studies show th[at bulbar] acidification abolishes acid responses to small doses of insulin (Nilsson 1969 a) [or to] short periods of sham feeding (Nilsson 1969 b), whereas acid responses to large [doses of] insulin (Nilsson 1969 a) or to prolonged sham feeding (Nilsson 1969 b) are only [partially] inhibited. It was suggested (Nilsson 1969 c) from those observations (Pevner and [Gross-]man 1955 Olbe 1964 Nilsson 1969 a and b) that the bulbar inhibitory mechanis[m does] not inhibit acid responses to stimulation under conditions in which the parietal c[ells may] be subjected to a strong direct cholinergic excitation.

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Acta physiol. scand. 1975. 95. 263–269

From the Department of Physiology and Biophysics, University of Lund, Sweden

Relative Contribution of Superficially Bound and Extracellular Calcium to Activation of Contraction in Isolated Rat Portal Vein

By

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Abstract

SIGURDSSON, S. B., B. UVELIUS and B. JOHANSSON. *Relative contribution of superficially bound and extracellular calcium to activation of contraction in isolated rat portal vein.* Acta physiol. scand. 1975. *95*. 263–269.

Spontaneous electrical and mechanical activity of the isolated rat portal vein is abolished after only 3 min in nominally Ca-free medium, and after 5–6 min there is no contractile response to depolarizing (122 mM K⁺) Ca-free solution. In the present study we have examined the electrical and mechanical responses of the portal vein to depolarization with simultaneous readministration of Ca^{2+} (2.5 mM) after periods of variable length in Ca-free standard solution. After 30 to 60 min of Ca depletion a slow contraction occurred in response to the high-K⁺ solution with 2.5 mM Ca^{2+}. When the period in Ca-free medium was reduced below 30 min an early faster phase appeared in the contracture response, and this phase was more rapid the shorter the time of Ca depletion. It is suggested that the slow contracture obtained after 30 min or more uses mainly extracellular Ca for activation and that the faster phase seen after shorter periods of Ca depletion is due to release of superficially bound Ca. This latter pool of tissue bound Ca does not alone produce contraction in response to depolarization, suggesting that extracellular Ca is required to trigger the release perhaps through a regenerative process.

It is generally agreed that Ca^{++} is necessary for contraction in smooth muscle as well as in other contractile systems. However, the relative importance of different possible sources of activator calcium in smooth muscle is still a matter of debate. With regard to vascular smooth muscle it has been found that certain preparations of isolated blood vessels, *e.g.* the rabbit aorta, maintain their ability to contract in response to various stimulants even after quite long periods in calcium-free solutions (Bohr 1963). This could indicate that in these vessels tissue bound Ca may be released and utilized for contraction. On the other hand, there are other preparations of vascular smooth muscle such as the rat portal vein, which lose their responsiveness after only a few minutes in Ca^{++}-free solution (*e.g.* Johansson 1974, see also below). It appears therefore that activator calcium in these tissues is more intimately related to extracellular Ca, but the engagement of bound fractions can by no means be excluded

NILSSON, G and S. J RUNE, Failure of high pCO_2 in the duodenal bulb to inhibit gastric acid [illegible]. *Acta physiol. scand.* 1971 *81* 349–354.

OLBE, L., Effect of resection of gastrin releasing regions on acid response to sham feeding and [illegible] hypoglycemia in Pavlov pouch dogs. *Acta physiol. scand.* 1964. 62. 169–175.

PEVSNER, L. and M L GROSSMAN, The mechanism of vagal stimulation on gastric acid secretion. *Gastroenterology* 1955 *28* 493–499

SJÖDIN L., Potentiation of the gastric secretory response to sham feeding in dogs by [illegible] and pentagastrin. *Acta physiol. scand.* 1972. *85* 24–32.

SJÖDIN, L. and G NILSSON Plasma gastrin levels and gastric acid secretion in dogs during [illegible] of Urecholine. *Scand. J Gastroent* 1974 *9* 747–750.

SNEDECOR, G W and W G COCHRAN, *Statistical methods.* Iowa State Press. Ames Iowa. 19[illegible]

UVNÄS, B., The part played by the pyloric region in the cephalic phase of gastric secretion. *Acta physiol. scand.* 1942. *4* suppl. 13.

WORMSLEY K. G Response to duodenal acidification on man II. Effects on the gastric secretory response to pentagastrin. *Scand. J Gastroent* 1970. *5* 707 215.

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…pontaneous electrical and mechanical activity of the isolated rat portal vein is abolished after only … min in nominally Ca-free medium, and after 5–6 min there is no contractile response to depolarizing … 2 mM K^+), Ca-free solution. In the present study we have examined the electrical and mechanical …ponses of the portal vein to depolarization with simultaneous readministration of Ca^{2+} (2.5 mM) after …riods of variable length in Ca-free standard solution. After 30 to 60 min of Ca depletion a slow contrac…re occurred in response to the high-K solution with 2.5 mM Ca^{2+}. When the period in Ca-free medium … reduced below 30 min an early faster phase appeared in the contracture response, and this phase was …ore rapid the shorter the time of Ca depletion. It is suggested that the slow contracture obtained after … min or more uses mainly extracellular Ca for activation and that the faster phase seen after shorter …riods of Ca depletion is due to release of superficially bound Ca. This latter pool of tissue bound Ca does …ot alone produce contraction in response to depolarization, suggesting that extracellular Ca is required to …igger the release perhaps through a regenerative process.

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The present study is an attempt to obtain information on the different sources of [illegible] Ca in portal vein. Some of the results have been reported in preliminary form (U[illegible] Sigurdsson and Johansson 1974)

Materials and methods

Experiments were performed on 40 portal vein preparations from rats of the Sprague-Dawley [illegible] ing 200–300 g. The animals were killed by a blow on the neck and the portal veins were dissected [illegible] experiments in which only isometric tension was recorded, the length of the preparations was [illegible] These were mounted in a 50 ml mantled organ bath and connected to a Grass FT03 force trans[illegible] a passive tension of about $4 \cdot 10^{-3}$ N. The experiments with simultaneous recording of electrical and [illegible] chanical activity were carried out with the sucrose gap apparatus using longer preparations of [illegible] mesenteric vein (see Axelsson *et al.* 1967). The electrical signals were amplified and recorded [illegible] as described by Johansson and Mellander (1975).

The bathing medium was a standard Tris-buffered solution ("Na-tris") of the following composition [illegible] mM NaCl 1?0, KCl 6.0, $MgCl_2$ 1.[illegible], glucose 11.5, tris(hydroxymethyl)aminomethane (Trizma [illegible] Chemical Co) 30. The pH was titrated to 7.4 with 1 M HCl. The temperature in the bath was held [illegible] To this nominally Ca-free solution various amounts of $CaCl_2$ were added giving Ca^{++} concentrations 0.5 1.0, 2.5 and 5.0 mM respectively. The depolarizing solutions ("K-tris") had the same composition [illegible] above, except that all NaCl was substituted with equimolar amounts of KCl. All solutions [illegible] bubbled with 100 % O_2.

Before the actual experiment started the muscles were allowed to accommodate for at least [illegible] Na-tris with 2.5 mM Ca.

Results

Fig. 1 A shows the spontaneous isometric contractions (upper tracing) and an AC sucrose gap recording of electrical activity (lower tracing) from a rat portal vein in "normal" [illegible] solution. After the one hour accommodation period, this superfusion medium was replaced by Ca-free Na-tris solution. Electrical and mechanical activity then rapidly disappeared. Six min later (Fig. 1 B) the muscle was transferred to K-tris solution with 2.5 mM Ca. This caused an early fast increase in tension associated with the appearance of [illegible] spike activity. When the contraction reached near maximum the spikes decreased in amplitude and number and finally disappeared. The later sustained contracture was associated with maintained membrane depolarization (not seen in the present AC recording). When the time in Na-tris Ca-free solution preceding the switch to K-tris with 2.5 mM Ca^{++} was increased to 10 min (Fig. 1 C) and 20 min (Fig. 1 D), respectively, the rate of rise of the contractile force became successively slower although the final contracture amplitude was unaltered. After 10 min only few and after 20 min no spikes were seen.

The differences in time course of K contractures due to different durations of the preceding Ca^{++} depletion were studied systematically in mechanical experiments with recording of isometric force. Original recordings from one such experiment are exemplified in Fig. 2. When the muscle, after 30 min in N-tris Ca free solution was placed in K-tris with 2.5 mM Ca^{++} (Fig. 2 A), a slowly developing contracture of the same type as in Fig. 1 D was obtained. On the other hand, when the muscle was transferred to the depolarizing solution already after 6 min, the development of tension was much faster although the final tension amplitude was unchanged (Fig. 2 B). If Ca-free depolarizing solution was administered after 6 min [illegible]

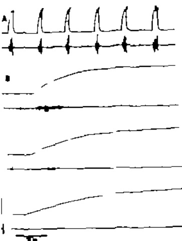


Fig. 1

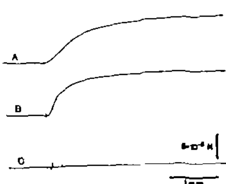


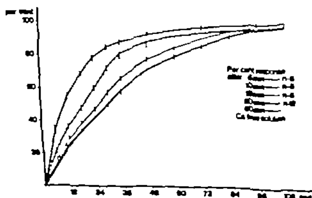
Fig. 2

A. Normal spontaneous activity of the rat portal vein recorded in sucrose gap apparatus. Upper mechanical activity lower tracing electrical activity (AC-recording, see text). B–D Effect of K-tris 5 mM Ca on the portal vein after exposure for 6 min (B) 10 min (C) and 20 min (D) in Ca-free solution.

Mechanical response to K-tris with 2.5 mM Ca after the muscle had been in Ca-free Na-tris for (A) Response to K-tris with 5 mM Ca (B) and to K-tris with 0 mM Ca (C) after 6 min in Ca-free solution.

... Na-tris no development of tension was seen (Fig. 2 C). Thus depolarization without ...taneous readministration of calcium failed to produce contraction already after short ...ds of preceding Ca depletion.

...e diagram in Fig. 3 summarizes the differences in time course of responses to K-tris ...5 mM Ca++ after 6, 10, 15 30 and 60 min of calcium depletion as obtained in muscles ...h had previously accommodated in Na-tris with 2.5 mM Ca++ for 30 min. Tension as a ...tion of time is expressed in per cent of the ultimate contracture level for each individual



...3 Comparison of the time ...se of contractile responses ...ced by K-tris ...th 2.5 mM ...after various times in Ca-free ...-tris solution. Contraction amplitude after 120 ...s set to 100%. ...rtical bars represent S.E.

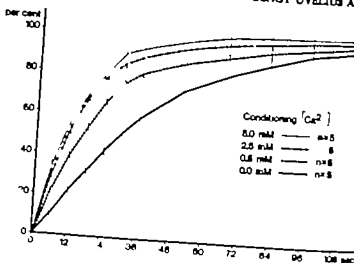


Fig. 4. Comparison of course of contracture K-tris with 2.5 mM Ca which had accom... solutions with various ... centrations. Before the ... ture the muscle was ... Na-tris solution for 6 m... traction amplitude after ... set to 100 ... Vertical ... resent S.E.

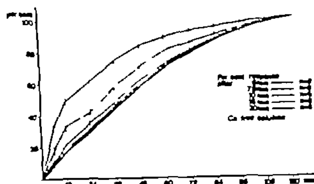
preparation. As is seen in the figure, those muscles which had been in Ca-free Na-tris ... for 30 and 60 min, respectively developed tension at the same slow rate. Those ... been in this solution for lesser periods showed a faster increase, steeper the shorter ... in Ca-free solution.

A separate series of experiments was performed in order to study the influence ... tions in the Ca concentration during the accommodation period on the contrac... sponse after a given time of Ca depletion. The muscles were allowed to accomm... described earlier for 30 min in Na-tris solution but now with the following concen... of Ca 0 0,5 2.5 and 5 0 mM After that period they were placed in Ca-free solu... 6 min and then exposed to K-tris with 2.5 mM Ca. The results are summarized ... It is seen that as the Ca concentration in the accommodating solution was increase... 0 to 2.5 mM a faster tension development occurred in the K-tris 2.5 mM Ca solu... further increase of Ca in the accommodating solution to 5 mM had no additional ef...

The results shown in Fig. 3 and 4 indicated that the fast component of the con... response was dependent on Ca accumulated in the preparation during the preced... commodation period. To study whether such accumulation could occur also in th... polarized state the following experiments were performed. The muscles were pla... before for 30 min in Na-tris with 2.5 mM Ca. They were then transferred to Ca-free N... for 30 min followed by exposure to K tris with 2.5 mM Ca for 4 min. Thereafter they ... placed for various periods (6, 7.5 10, 15 and 30 min) in Ca-free Na-tris solution where ... rapidly relaxed. They were then allowed to contract again in K-tris with 2.5 mM Ca ... results are shown in Fig. 5 Variations in the time course of the second contracture resp... were expected to reflect differences in the loss of calcium which had possibly accumul... during the first contracture period. No significant difference in time course is seen betw... the curves for 10, 15 and 30 min depletion but the results after 7.5 and 6 min show that ... phase occurs also under the conditions of these experiments.

Discussion

Spontaneous electrical and mechanical activity in the isolated portal vein is abolished ... only 2–3 min in nominally Ca-free solution (e g Johansson 1974) and the contractile ...



Comparison of the time of contractile responses d by K-tris solution with M Ca in muscles which had n Ca-free Na-tris solution ous times after an earlier exposure to K-tris ith M Ca. Contraction am- after 120 s set to 100 al bars represent S.E.

se to K -depolarization without simultaneous administration of exogenous Ca^{++} dis- ars after 5-6 min (Fig. 2). This implies that the concentration of free calcium in the cellular space decreases rapidly in the nominally Ca-free medium since earlier studies shown that spontaneous activity and K contracture require threshold concentrations 5 and 0.1 mM Ca^{++} respectively (Uvelius, Sigurdsson and Johansson 1975, Biamino and nsson 1970). The difference in time course of the contractile responses to K-tris with mM Ca^{++} after for instance 6 and 30 min of Ca depletion (Fig. 1 and 2) cannot therefore lue to differences in the amount of free Ca remaining in the extracellular fluid. It is more ly that a superficially bound Ca fraction is mobilized after the short periods of depletion that it contributes to the contractile activation and to the spike phenomenon which en accompanies the fast phase of the contraction. Calcium in the extracellular fluid is fully necessary to trigger the release of this bound fraction at the depolarization of the , after 6 min of depletion, $[Ca^{++}]_o$ has dropped below the level required (Fig. 2 C).

Within 6 to 30 min of preceding Ca depletion the response to K depolarization with ultaneous administration of exogenous Ca^{++} appears to be due to a combined release of from the superficial bound pool (fast phase of the response) and influx of extracellular $^{++}$ (slow phase). The identical shape of the responses after 30 and 60 min defines the slow ase. The reasons for believing that this phase represents influx of extracellular Ca are that amplitude is greatly dependent on $[Ca^{++}]_o$ and that it does not change even with prolonged posure to Ca-free solution (Biamino and Johansson 1970, Hellstrand, Johansson and ngberg 1972, U elius *et al.* 1975). The portal ein undoubtedly contains deeper cellular ols of Ca which seem ery resistant to depletion and which, in the absence of extracellular can support contraction only when exceptional modes of stimulation are applied nderson *et al.* 1974)

The amount of calcium which can be liberated from the superficial binding sites to give e fast phase of the contraction varies not only with the time in the Ca-depleting medium Fig. 3) but also with the $[Ca^{++}]_o$ in the preceding accommodation period (Fig. 4). It appears hat this pool is practically saturated at normal levels of $[Ca^{++}]_o$. Furthermore, the results own in Fig. 5 indicate that the superficial binding sites can accumulate calcium also when he cell is depolarized. The anatomical localization of these sites remains to be demonstrated,

but the basal membrane, the plasma membrane and perhaps the superficially located sarco-plasmic reticulum (Devine, Somlyo and Somlyo 1973) appear most likely

Other authors have previously distinguished between slow and rapid components of vascular responses to various stimuli and have given different interpretations to their findings. Bohr (1963) in a study of epinephrine stimulated rabbit aortic strips attributed the [illegible] in the fast and the slow phase of the contractile response to effects on membrane excitation and on excitation-contraction coupling, respectively More recently Sitrin and Bohr (1971) have ascribed fast and slow components in the responses of rabbit aortic and dog mesenteric artery strips to different sources of activator calcium. In a study of rabbit anterior mesenteric portal vein Collins, Sutter and Telser (1971) found two components in the response to noradrenaline and potassium and suggested that the early phase was related to liberation of superficially bound calcium and the later one to influx of extracellular calcium. The constrictor response of the isolated rabbit ear artery also shows a distinct rapid component followed by a slow phase. The first component was attributed by Bevan and Waterson (1971) to myogenic spread of excitation from the surface layer of smooth muscle cells and they considered the second one related to saturation of the extracellular space with the stimulating agent (norepinephrine) Steinsland, Furchgott and Kirpekar (1973), on the other hand, considered the biphasic response of the rabbit ear artery to reflect different sources of activator calcium Evidence has been presented indicating that different stimuli e.g. noradrenaline and potassium may differ in their relative utilization of different calcium pools in vascular smooth muscle (e.g Peiper Griebel and Wende 1971 van Breemen et al. 197[illegible] Golenhofen, Hermstein and Lammel 1973).

It is somewhat difficult to make a direct comparison between our own results and those of earlier investigators mentioned above due to the different preparations and modes of stimulation. Our own results and interpretations are in good agreement with those of Sitrin and Bohr (1971) Collins *et al* (1971) and Steinsland *et al* (1973). The new point of the findings is the indication that extracellular Ca is required for release of Ca from the superficial binding sites perhaps through a regenerative mechanism. This is suggested by the finding that this pool contains effective amounts of Ca after more than 15 min in Ca-free solution (Fig. 3), but that it already after 6 min has lost its ability to alone support contraction response to depolarization.

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References

ANDERSSON, C., P HELLSTRAND, B. JOHANSSON and A. RINGBERG, Contraction in venous smooth muscle induced by hypertonicity Calcium dependence and mechanical characteristics. *Acta physiol. scand.* 1974, 90 451–461

AXELSSON, J B. WAHLSTRÖM, B. JOHANSSON and O JONSSON Influence of the ionic environment on spontaneous electrical and mechanical activity of the rat portal vein *Circulat Res.* 1967 21 609–618.

BEVAN, J. A. and J G WATERSON, Biphasic constrictor response of the rabbit ear artery *Circulat. Res.* 1971 28 655–661

...o, G. and B. Johansson, Effects of calcium and sodium on contracture tension in the smooth muscle ...e rat portal vein. *Pflügers Arch. ges. Physiol.* 1970. *321* 143–158.

...D. F Vascular smooth muscle: Dual effect of calcium. *Science* 1963. *139* 597–599

...a, G. A., M. C. Sutter and J C. Towers, Calcium and contraction in the rabbit anterior mesenteric ...al vein. *Canad. J. Physiol. Pharmacol.* 1971 *50*. 289–299.

...t, C. E., A. V Somlyo and A. P Somlyo, Sarcoplasmic reticulum and mitochondria as cation ac...ulation sites in smooth muscle, *Phil. Trans. B.* 1973. *165*. 17–23.

...orn, K., N. Hasselton and E. Lammel, Membrane potential and contraction of vascular smooth ...cle (portal vein) during application of noradrenaline and high potassium, and selective inhibitory ...ts of Iproveratril (Verapamil). *Microvasc. Res.* 1973. *5* 73–80.

...rland, P B. Johansson and A. Ringberg, Influence of extracellular calcium on isometric force ...velocity of shortening in depolarized venous smooth muscle. *Acta physiol. scand.* 1972. *84*. 528–537

...son, B., Determinants of vascular reactivity *Fed. Proc.* 1974. *33*. 121–126.

...son, B. and S. Mellander, Static and dynamic components in the vascular myogenic response to ...ive changes in length as revealed by electrical and mechanical recordings from the rat portal vein. ...culat. Res. 1975. *36*. 76–83.

...t, U., L. Goerdel and W Wende, Activation of vascular smooth muscle of rat aorta by noradrenaline ...depolarization: Two different mechanisms. *Pflügers Arch. ges. Physiol.* 1971. *330*. 74–89.

...t, M. D. and D P Bohr, Ca and Na interaction in vascular smooth muscle contraction. *Amer J ...siol.* 1971. *220*. 1124–1128.

...rland, O. S., R. F Furchgott and S. M. Kirpekar, Biphasic vasoconstriction of the rabbit ear ...ery. *Circulat Res.* 1973. *32*. 49–54.

...ur, B S. R. Sigurdsson and B. Johansson, Role of superficially bound Ca for potassium contrac...n in rat portal vein. *Acta physiol. scand.* 1974. *91* 2 A.

...ur, B S. R. Sigurdsson and B. Johansson, Strontium and barium as substitutes for calcium on ...trical and mechanical activity in rat portal vein. *Blood Vessels* 1974. *11* 245–253.

...Breemen, C., B R. Farinas, P Gerba and E. D McNaughton, Contraction coupling in rabbit ...ta studied by the lanthanum method for measuring cellular calcium influx. *Circulat. Res.* 1972. *30*. ...-54.

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From the Department of Pharmacology University of Lund, Sweden

Inhibition of Induced Pinocytosis in Amoeba proteus by Membrane Stabilizing Drugs

By

J -O JOSEFSSON G JOHANSSON and S. ELISABETH HANSSON

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Abstract

JOSEFSSON, J -O G JOHANSSON and S. ELISABETH HANSSON *Inhibition of induced pin[ocytosis] in Amoeba proteus by membrane stabilizing drugs.* Acta physiol. scand. 1975 95, 27[0]

The effect of membrane stabilizing drugs on cation induced pinocytosis was studied in *Amoeba* [*proteus*]. Initially the presence of local anesthetic drugs during a pinocytosis cycle had a stimulating effect on c[hannel] formation, however the capacity to develop pinocytotic channels was reversibly inhibited after a p[re]treatment with these drugs. Imipramine, vinblastine and the phenothiazines had effects similar t[o local] anaesthetics. The local anesthetics inhibited pinocytosis in the following order: dibucaine tetr[acaine] bupivacaine > lidocaine procaine, and the phenothiazines: thioridazine > prochlorperazine ch[lorpro]mazine > promethazine. Pinocytosis, when induced by Na or tris, was more affected by the drug[s and] calcium binding agents than pinocytosis induced by K After pretreatment with inhibitory conc[entrations] of dibucaine (3 10^{-4} M) the depolarization of the membrane and the conductance increase during [pinocy]tosis were normal, while the increase of oxygen uptake during the pinocytosis cycle was abolished. A[ddition] of Ca^{++} before, during or after dibucaine treatment decreased the effect of the drug. Conversely, [in dibu]caine-treated cells, cation induced pinocytosis was less inhibited by Ca^{++} than pinocytosis in norm[al cells]. Addition of EDTA to the inducing solutions potentiated the inhibitory effect of the drug. It is su[ggested] that these drugs release Ca^{++} from the cell surface and at higher concentration or after prolonged tr[eatment] time interfere with a Ca^{++} mechanism which couples the membrane and contractile system in th[e cyto]plasm.

The pinocytosis mechanism in *Amoeba proteus* has been suggested to be an excita[tion–] contraction process in which Ca^{++} couples the excitation (depolarization) of the mem[brane] to a channel forming system in the cytoplasm (Josefsson 1968). On the other hand Ca^{++} producing increased membrane resistance and increased membrane potential, i[nhibits] induction of pinocytosis. Monovalent inorganic cations have a Ca^{++} dependent depol[arizing] action wich parallels their ability to induce invagination of the cell membrane. (Brau[n and] Freeman 1967 Josefsson *et al* 1975) In the past years an important and specific c[lass of] agents characterized pharmacologically as membrane stabilizers has been elucidated [to] interfere with physiological functions which are dependent on the movement of [Ca^{++}] consequently in an excitation–contraction mechanism the local anesthetics may int[erfere] with the coupling between excitation and contraction (Feinstein [...] laustein and

...n 1966 a) as ... ell as with the initial excitatory event (which in amoeba is Ca^{++} dependent). ... the present report the inhibiting effect of a number of membrane stabilizing drugs on the ...ocytosis induced by K... and tris is described and an attempt is made to define the site of action of these drugs.

Methods

...oeba proteus, fed on *Tetrahymena pyriformis*, were starved in Prungsheim solution three days before an ...periment. The composition of the culture medium (Prungsheim) was (mM): Na 0.22, K 0.35, Ca^{++} ..., Mg^{++} 0.08, Fe^{++} 0.007 HPO_4^{--} 0.11, Cl^- 0.35, NO_3^- 1.70, SO_4^{--} 0.09 pH 7.0. All studies were made ... room temperature (22–25°C). Inducing salts were chlorides of Analar grade obtained from the British ...ug Houses, Poole, England. Glass distilled water ... as used throughout the experiments.

Determination of pinocytosis was made at 23°C (temperature being measured on the microscope stage) ...cording to ... modification of ... technique by Josefsson (1968). Exchange of extracellular medium by the ...ducing solution was made ... th the amoebae (200–400) on glass microscope slides. The total volume used ... 2.5 ml and the exchange was carried out in three steps and completed within one minute. The efficacy ...f exchange was, as calculated from dilution of 3-O-methyl-D-glucose (methyl ...C) to be better than 1 : 10⁴ ...ch amoeba was then inspected for pinocytotic channels during 30 sec only. This procedure was continued ...il 40 amoebae had been observed. Dividing the sum of the number of channels by 40 (the number of ...servations) gives the mean value of channels observed in an average amoeba during the first 20 minutes ...f the pinocytosis cycle. This value is designated ... channels ... in the graphs and used as an index of pinocy...tic activity.

Preincubation of cells with drugs was carried out in moist chambers. The cells were adherent to micro...cope slides and the culture solution ... as exchanged for the solution containing the drug. The drugs ... ere ...pplied either dissolved in distilled water, tris buffer or in ... Prungsheim solution from which divalent ...tions had been omitted (CFP = calcium free Prungsheim). Cells used as controls were preincubated in CFP solution (0.11 mM Na_2HPO_4 and 0.35 mM KCl at pH 7.0) or in 0.3 mM NaCl. The salts used were ...f analytical grade. pH ... as adjusted with HCl or LiOH and determined with an expanded scale electro...meter using Radiometer electrodes.

... Salts of EGTA (1,2-bis 2-amino-ethoxyethane tetraacetic acid) were used at pH 7.0 to obtain extra...cellular media of low Ca^{++} concentrations. A conditional formation constant of the Ca-EGTA complex (pH 7.0, ionic strength 0.1 and 20°C) was calculated to be 10⁶·... from log K_{CaEGTA} 11.0 and dissociation ...nstants for the chelator given by Schwarzenbach (1960). The concentrations of the free ionized Ca^{++} was ...calculated according to the formula

$$pCa = 6.7 + \log\left\{\frac{[EGTA \quad added]}{[CaCl_2 \quad added]} - 1\right\}$$

...spiratory rates in cell populations were measured at 22°C with ... Clark-type polarographic electrode ...held ... lucite plunger ... hich fitted tightly into ... 6 ml sample chamber equipped ... ith ... magnet bar. The ...rrent ... as measured ... ith an YSI model 53 oxygen monitor (Yellow Springs Instrument Co. ... USA) and ...corded on ... strip chart recorder (Servogor Goertz, Austria). Aliquots containing about 50 000 cells were ... taken from ... cell suspension agitated ... ith ... magnetic stirrer. The agitated suspension was centrifuged at ... low speed (300 g ... min) in order to separate the cells and incubated ... ith the drug solution. After 30 min ...f incubation ... ith the drug, the cells were carefully washed ... ith Prungsheim solutions before they were transferred to the electrode chamber. The oxygen consumption was registered in arbitrary units from the ...slope of the graphs. Measurements were started ... hen the cells had been in the solution for 5 min. Measure...ments of oxygen consumption ... ere also made manually on single cells with the cartesian diver technique ...ferred ... to the detailed description by Hansson *et al.* (1968).

The calcium content of cells and external media was measured with ... Unicam SP 90 Atomic absorption ...spectrophotometer. Cells ... ere homogenized with ultrasound (Branson Sonifier) and $LaCl_3$ (final concen...tration 0.5 mM) ... as added to overcome the suppression of the calcium absorption by organic material. To separate the extracellular calcium from the cellular calcium the cell suspension was washed in the ...rrhage ... ith 1 mM NaCl pH 6.0 and incubated at room temperature for 30 min with 1 mM $LaCl_3$. The ca...lcium content of the extracellular solution so obtained ... as taken as ... measure of the calcium bound ... surface.

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Inhibition of Induced Pinocytosis in Amoeba proteus by Membrane Stabilizing Drugs

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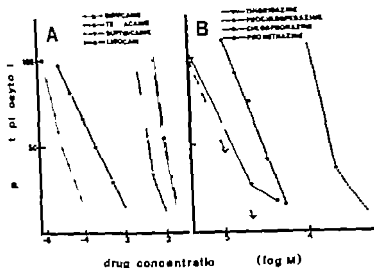
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Abstract

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The effect of membrane stabilizing drugs on cation induced pinocytosis was studied in *Amoeba pro…* Initially the presence of local anesthetic drugs during a pinocytosis cycle had a stimulating effect on cha… formation, however, the capacity to develop pinocytotic channels was reversibly inhibited after a perio… treatment with these drugs. Imipramine, vinblastine and the phenothiazines had effects similar to l… anesthetics. The local anesthetics inhibited pinocytosis in the following order: dibucaine > tetracai… bupivacaine > lidocaine procaine, and the phenothiazines: thioridazine prochlorperazine chlor… mazine > prometazine. Pinocytosis, when induced by Na or tris, was more affected by the drugs an… calcium binding agents than pinocytosis induced by K. After pretreatment with inhibitory concentra… of dibucaine ($3 \cdot 10^{-4}$ M) the depolarization of the membrane and the conductance increase during pin… tosis were normal, while the increase of oxygen uptake during the pinocytosis cycle was abolished. Addi… of Ca^{++} before, during or after dibucaine treatment decreased the effect of the drug. Conversely, in d… caine treated cells, cation induced pinocytosis was less inhibited by Ca^{+} than pinocytosis in normal c… Addition of EGTA to the inducing solutions potentiated the inhibitory effect of the drug. It is sugge… that these drugs release Ca^{++} from the cell surface and at higher concentration or after prolonged incubat… time interfere with a Ca^{++} mechanism which couples the membrane and contractile systems in the c… plasm.

The pinocytosis mechanism in *Amoeba proteus* has been suggested to be an excitatio… contraction process in which Ca^{+} couples the excitation (depolarization) of the membra… to a channel forming system in the cytoplasm (Josefsson 1968). On the other hand exc… Ca^{++} producing increased membrane resistance and increased membrane potential, inhib… induction of pinocytosis. Monovalent inorganic cations have a Ca dependent depolariz… action wich parallels their ability to induce invagination of the cell membrane. (Brandt a… Freeman 1967, Josefsson *et al.* 1975.) In the past years an important and specific class… agents characterized pharmacologically as membrane stabilizers has been elucidated. Th… interfere with physiological functions which are dependent on the movement of Ca… consequently in an excitation-contraction mechanism the local anesthetics may interfe… with the coupling between excitation and contraction (Feinstein 1964, Blaustein…



Inhibition of pinocytosis after pretreatment of the cells for 25 min with local anesthetics (Fig. 1 A) phenothiazines (Fig. 1 B) at pH 6.0 dissolved in 0.3 mM tris-HCl. Pinocytosis was induced by 125 mM [illegible]Cl at pH 7.0. The pinocytosis intensity is given on the ordinate in percent of that induced in cells [illegible] with 0.3 mM tris-HCl with no drug present. The concentrations of the drugs are given on the abscissa. Each experimental point indicates a single measurement.

ciently at acid and alkaline pH. Tris induced pinocytosis was effectively prevented when ionized drugs were applied to the cells in solutions at pH 5.5–6.5. In a series of 10 expts. ...caine was found to be less effective at pH 7.2 than at pH 5.5 (Table I). The differences in potency were greater than the differences in protonation of the drug (pKa 8.5) at these pH values. These results suggest a pH-sensitive barrier for ionized species of the drugs in the cell membrane. By way of this mechanism, pH of the solution may influence the

Table I. Pinocytosis induced by 40 mM KCl at pH 5.7 or 100 mM tris HCl at pH 7.0 after pretreatment of the cells for 30 min with solutions of some organic bases at pH 5.5–5.7. All salts with the exception of prochlorperazine methanesulphonate were chlorides. Controls rated at 100 are carried out on aliquots of cells taken directly from the culture fluid. Relative intensity of potassium induced pinocytosis is given in percent as the mean of 2 to 4 expts. Tris induced pinocytosis is given in percent of controls ± S.E. of 10 expts.

Pretreatment		Conc. (M)	Inducer	Relative pinocytosis intensity
Thioridazine	pH 5.7	10^{-4}	40 mM KCl	68
Prochlorperazine	pH 5.7	10^{-4}	40 mM KCl	87
Chlorpromazine	pH 5.7	10^{-4}	40 mM KCl	128
Chlorpromazine	pH 5.7	10^{-4}	40 mM KCl	8
Promethazine	pH 5.7	10^{-4}	40 mM KCl	59
Dibucaine	pH 5.5	10^{-4}	40 mM KCl	39
Dibucaine	pH 5.5	$3 \cdot 10^{-4}$	40 mM KCl	34
Dibucaine	pH 5.5	$3 \cdot 10^{-4}$	100 mM tris HCl	18.3 ± 5.1
Dibucaine	pH 7.3	$3 \cdot 10^{-4}$	100 mM tris HCl	38.3 ± 8.0

For membrane potential measurement about 100 cells were transferred from the culture dish to a [illegible] locite chamber carefully washed with Pringsheim solution. The cells were allowed to adhere to the [illegible] surface of the chamber and after 10 min the culture solution was substituted by the addition of [illegible] test solution. Conventional glass capillary microelectrodes with tip potentials not exceeding [illegible] used for the measurement of membrane potentials. Such electrodes were obtained by selection and [illegible] times supported by the addition of 0.01–0.05 mM thorium nitrate to the electrolyte used for filling [illegible] electrode. For continuous longtime measurements electrodes filled with 0.3 M KCl without the addition [illegible] thorium were used. The 3 M KCl electrodes had about 20 MΩ resistance when measured in 3 M KCl [illegible] differences in membrane potential due to thorium present in the electrode were observed when the [illegible] obtained with different types of electrodes were compared. All stimulating electrodes were filled [illegible] 3 M KCl. The measurements of membrane potential, and input resistance were made in the rear [illegible] part of the cell. The membrane resistance was calculated from the current necessary to be injected to [illegible] the membrane potential match a command signal applied to the cell in a voltage clamp circuit. The method has been described elsewhere (Josefsson *et al.* 1975).

Results

Preincubation of cells with local anesthetics in the culture fluid, or addition of local anesthetics of the solution of the chemical inductor did not, in general inhibit the formation [illegible] channels. Preincubation with the same anesthetic drug concentration but in a Ca^{++}-free medium (CFP or 0.3 mM NaCl) was, however inhibitory At the end of a typical period [illegible] pretreatment (0.3 mM dibucaine for 30 min at pH 5 6) attachment of the cells to the glass surface was reduced and streaming of the cytoplasm was slow The contractile vacuole [illegible] swollen and the frequency of its contractions was decreased In high concentrations ([illegible] 25 mM) of the drugs pinocytosis cycles of low intensity were induced. The potent anesthetics dibucaine and tetracaine were less effective inducers than procaine and lidocaine. Like [illegible] cation induced pinocytosis, Ca^{++} inhibited that induced by local anesthetics.

Effects of pretreatment with membrane stabilizing drugs

Dose–response relationship Amoebae preincubated in solutions of local anesthetics w[illegible] found to develop few pinocytotic channels in response to a strong inducer such as 100 [illegible] tris-HCl at pH 7 0 The magnitude of pinocytosis blockade was dependent on the concentration of the local anesthetic, the length of the incubation period and on the specific potency of the drug. Dibucaine and tetracaine were effective at low concentrations while bupivacaine (Marcaine®) and lidocaine had weaker effects (Fig. 1 A) The phenothiazine drugs w[illegible] equal to or more potent than the local anesthetics. The former inhibited pinocytosis induced by 100 mM tris-HCl (Fig. 1 B) or 40 mM KCl (Table I) in the order thioridazine > prochlorperazine > chlorpromazine > promethazine. Generally all these drugs inhibited potassium-induced pinocytosis less efficiently than sodium or tris induced pinocytosis.

Effect of pH Since the outer surface of the membrane of the amoeba is a cation exchanger (Marshall and Nachmias 1965 Hendil 1971) the membrane stabilizers could be effective as their ionized (cationic) forms. This was studied by the use of benzocaine (pKa 3.2), which exists essentially as a non-ionized free base between pH 5 and pH 8 and two quaternary local anesthetic amines (Fig. 2). In contrast to dibucaine (pKa 8.5) and the quaternary derivatives of both promethazine (N-hydroxy-ethylpromethazine) and lidocaine (QX 572) (for chemical structure see Johnson and Schwartz 1969) benzocaine prevented pinocytosis

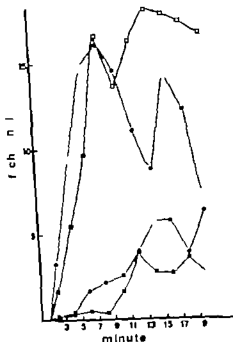


Fig. 4. Time course of channel formation during pinocytosis induced by 100 mM tris at pH 7.0. The shape of the curves varied according to the treatment of the cells in the period preceding induction of pinocytosis. Open symbols indicate that cells were tested directly from the culture solution (squares) or after pretreatment with 0.3 mM NaCl for 30 min (circles). Solid symbols indicate that cells were pretreated with 0.3 mM dibucaine at pH 5.6 (squares) or pH 7.0 (circles). Means of 7–10 expts. were used to make each curve.

Lower concentrations of dibucaine, required longer preincubation periods to obtain maximal effect. The period of complete blockade of pinocytosis following pretreatment was generally of short duration (Fig. 3) provided the cells were washed in calcium containing media.

Time course of channel formation after treatment with dibucaine. After dibucaine treatment the onset of channel formation was slow. The time dependence of channel formation following application of 100 mM tris pH 7.0 is given in Fig. 4. Cells pretreated in 0.3 mM dibucaine for 30 min at two different pH values were compared with normal cells and cells preincubated in 0.3 mM NaCl. The control cells formed maximal number of channels during 8 min and 15 min of the cycle, while channel formation induced after treatment with the local anesthetic was *depressed during the first 10 min of the cycle. The partial recovery of* channel formation at the end of the cycle may be analogous to recovery of pinocytosis shown in Fig. 3. In the Ca^{++}-deficient, Na -treated cells the channels appeared earlier in the pinocytosis cycle than in the control cells.

Altered dose-response relationship for inducers after treatment with dibucaine. Dose-response relationships for pinocytosis inductors applied to anesthetized cells were investigated to elucidate the mechanism of inhibition. Fig 5 A and B show respectively the blockade of pinocytosis induced by K and tris following pretreatment with dibucaine. With both inducers a non-competitive blockade of pinocytosis was noticed when dibucaine was applied to the

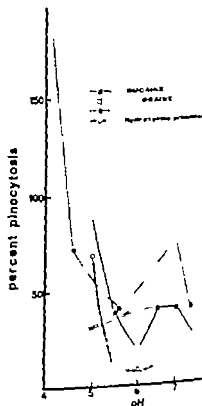


Fig. 2. Intensity of pinocytosis induced by tris (100 mM pH 7.0) as a function of the pH (abscissa) of the drug solution after pretreatment for 30 min with four different inhibitors. The intensity of pinocytosis is given as a percentage of the control which was pretreated with 0.3 mM NaCl at the respective pH. The following concentrations and incubation periods were used dibucaine 0.1 mM 60 min, benzocaine 4.7 mM 10 min N-hydroxyethylprometazine and QX 572 0.3 mM for 30 min.

effectiveness of cations to induce pinocytosis. In all these experiments 100 mM tr pH 7 0 was the inducer similar results were obtained with 25 mM KCl.

Time course and reversibility of the effect of dibucaine The effect of preincubatio with local anesthetics on the subsequent blockade of tris induced pinocytosis was inves with dibucaine at pH 5.5 (Fig. 3). Preincubation for a period of at least 10 min was n to produce a complete blockade of pinocytosis when the dibucaine concentration was (

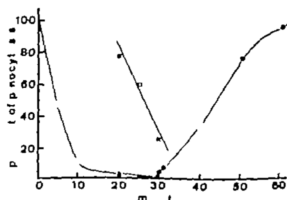


Fig. 3. Inhibition and recovery of pinocytosis induced by tris (100 mM, pH 7.0) after incubation in dibucaine 0.3 mM at pH 5.5. The intensity of pinocytosis is given on the ordinate in percent number of channels developed in the untreated control. Solid triangles indicate pinocytosis inte treatment of the cells with dibucaine 0.3 mM for 5, 10, 20 and 30 min. Open squares g e the al treatment with 0.1 mM dibucaine fo 20, 25 and 30 min. Solid circles indicat the recovery in Pr solution after an incubation period of 30 min in 0.3 mM dibucaine.

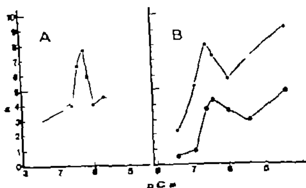


Fig. 7 A. Effect of ambient Ca^{++} concentration during potassium (25 mM pH 7.0, circles) and sodium induced pinocytosis (100 mM pH 7.0, squares). The Ca^{++} concentrations are adjusted with an EGTA—Ca^{++} buffer. The calculated Ca^{++} concentration is given as pCa on the abscissa.

Fig. 7 B. Pinocytosis induced by KCl (20 mM pH 7.0) in cells preincubated for 30 min at pH 5.6 in 0.3 mM NaCl (solid circles) or 0.3 mM dibucaine (open circles). In addition to inhibition at all Ca^{++} concentrations the latter curve displayed a shift to the right from the control curve.

effect on pinocytosis (induced by tris or K), sodium thiopenthal (1 mM), and tetrodotoxin (0.01 1 μg/ml) were found to be without effect when applied 30 min before, or during pinocytosis. The organic bases, imipramine and desmethylimipramine, however possessed an inhibitory effect comparable in strength to that of dibucaine. Likewise vinblastine sulphate (0.3–0.5 mg/ml pH 5.6) applied to the cells in CFP 30 min before induction inhibited tris induced pinocytosis by 95 percent (Fig. 6 A) and K -induced pinocytosis by 75 percent. Recovery from inhibition occurred when the cells were transferred to Pringsheim solution (Fig. 6 B). Colchicine (up to 10 mM) slightly stimulated tris induced pinocytosis.

Effects of dibucaine pretreatment on pinocytosis in the presence of Ca^{++}. As described previously (Josefsson 1968), pinocytosis induced by low concentrations of monovalent cations is stimulated by small amounts of the Ca^{++} chelating agent EGTA, while a low pCa tends to block pinocytosis. It seemed that, by the use of a Ca^{++} buffer system it should be possible to find optimal Ca^{++} levels for different types of pinocytosis. Fig. 7 A illustrates such experiments for KCl and NaCl induced pinocytosis. When the Ca^{++} concentration of the inducing KCl solution (40 mM) was varied by the use of 5 mM Ca-EGTA buffer optimal pinocytosis was found at a calculated Ca^{++} concentration of about $10^{-6.9}$ M. In similar experiments (not shown) the shape of the pCa-pinocytosis curve was the same in both 10 and 20 mM KCl. With 100 mM KCl a second optimal Ca^{++} concentration at about pCa 5 was noted. The pinocytosis induced by NaCl (100 mM), appeared to be maximally stimulated at a still higher Ca^{++} concentration (Fig. 7 A).

Such experiments were also carried out on cells pretreated with 0.3 mM NaCl or dibucaine-HCl. It was found that the curve after dibucaine-HCl treatment (Fig. 7 B) describing potassium induced pinocytosis as a function of pCa was shifted towards higher Ca^{++} concentrations but retained the shape typical for cells pretreated with NaCl. These experiments might indicate that dibucaine depletes the cells of Ca^{++}. By measuring the Ca^{++} content of the extracellular medium (Fig. 8) it was confirmed, that dibucaine released more Ca^{++} from the

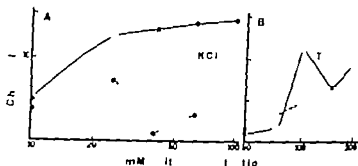


Fig. 5 A. Dose–response relationship for potassium induced pinocytosis in cells pretreated for 30 min with 0.3 mM NaCl pH 5.8 (solid line) or 0.3 mM dibucaine pH 5.6 (broken line).

Fig. 5 B. Dose–response relationship for tris induced pinocytosis in cells pretreated for 30 min at 0.1 mM NaCl (solid line) or in dibucaine 0.1 mM pH 5.6 (broken line).

cells 30 min before the experiment. In Fig. 5 A the intensity of pinocytosis induced by 10–100 mM KCl is given for cells pretreated with 0.3 mM of dibucaine or as a control with NaCl. Treatment with the latter solution did not prevent a normal pinocytosis cycle but appeared to abolish the "auto-inhibition" of high K^+-concentrations, as described earlier (Josefsson 1968). Treatment with dibucaine prevented channel formation in all KCl solutions and especially at 40 mM KCl where pinocytosis in normal cells is most intense. Compared to controls in 0.1 mM NaCl, dibucaine did not prevent pinocytosis in response to low concentrations of tris (Fig. 5 B). The pinocytosis induced by 100 mM and higher concentrations of tris was, however, markedly depressed.

Effects of some other drugs on the capacity for pinocytosis. In a survey for drugs with an

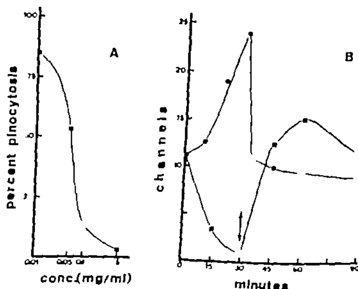
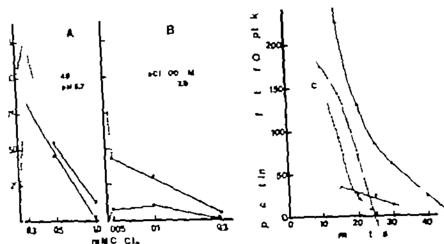


Fig. 6 A. Effect of pretreatment for 30 min with vinblastine sulphate on pinocytosis induced by 100 mM tris pH 7.0. The concentration of the drug, dissolved in CFP at pH 5.6, is given on the abscissa. The ordinate indicates the intensity of pinocytosis as percent of the control cells treated in CFP.

Fig. 6 B. Time course of inhibition of tris induced pinocytosis by 0.5 mg/ml vinblastine sulphate in CFP at pH 5.6 (squares). For comparison the pinocytosis intensity after treatment with CFP but without the drug is indicated by circles in the figure. Note that the intensity of pinocytosis increases in the course of treatment with the CFP solution. After 30 min of treatment (arrow) the cells were washed and transferred to Pringsheim solution. With both types of pretreatment normal pinocytosis was restored within 15 min.



9 Effect of dibucaine pretreatment on calcium inhibition of potassium (A) and sodium induced systems (B). The Ca^{++} concentration of the inducing solution is given on the abscissa. The pinocytosis activity given on the ordinate as percentage of the pinocytosis elicited by the inducers 40 mM KCl pH and 100 mM NaCl pH 7.0. Each experimental point indicates single measurement on normal cells (dots) and cells pretreated for 30 min with 0.3 mM dibucaine pH 5.6 (squares).

10. Rate of oxygen uptake after induction of pinocytosis (curve A, B and D) and after the exchange of two media (curve C). The measurements were made with Cartesian micro-divers at 25°C. Pinocytosis induced by 100 mM tris-HCl pH 7.0. Oxygen uptake is given as percentage increase from the steady rate of O_2 uptake measured for 10 min period before the introduction of the inducing solution at time. All curves start at ordinate zero. The manually controlled diver technique did not allow measurement to be made earlier than 10 min after the exchange of solution. *Curve C* indicates the normal transient increase in respiration following exchange (at zero time) of Pringsheim solution in the micro-diver. *Curve B* describes the respiratory increase in control cells during pinocytosis which follows upon exchange of 0.3 mM KCl for the inducing solution. *Curve A* represents conditions as for curve B with the exception that 0.3 mM dibucaine was present in the inducing solution. *Curve D* gives the increase of O_2 uptake during pinocytosis in cells pretreated for 30 min with 0.3 mM dibucaine. The conditions in A and D correspond to stimulated and inhibited pinocytosis respectively. Each experimental point is the mean of 5 experiments.

-ibiting action, the interaction between the two types of pinocytosis blockers was examined. The interference of dibucaine with Ca^{++} inhibited pinocytosis was investigated in 40 mM KCl (pH 5.7) or 100 mM NaCl (pH 7.0) (Fig. 9 A and B). No additive effects of the two types of inhibitors were observed. On the contrary dibucaine pretreatment partly counteracted the Ca^{++} inhibition of pinocytosis. The intensity of pinocytosis induced by 40 mM KCl in the presence of 0.5–1 mM $CaCl_2$ did not differ significantly in control cells and cells pretreated with dibucaine. Using NaCl (100 mM, pH 5.7 and 7.0) or tris HCl (100 mM pH 7.0) as inducing salts prevention of pinocytosis by dibucaine was, however only partially counteracted when Ca was added to the inducer.

Oxygen uptake after dibucaine treatment

During pinocytosis induced by several monovalent inorganic cations the oxygen uptake of the amoeba was increased (Hanston *et al.* 1968). This metabolic response to the pinocytosis inducer was reduced when Ca^{++} was added in concentrations which prevented pinocytosis. It was also found that mechanical manipulations of the cells as well as factors which stimulate channel formation increased the rate of respiration. The latter was measured with the

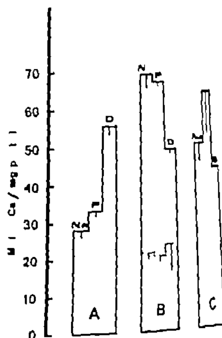


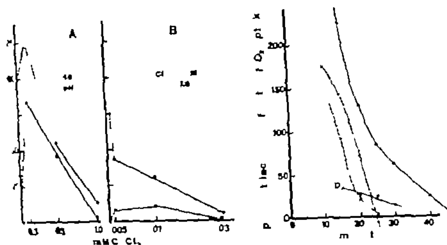
Fig. 8. Calcium in cells and media after incubation at pH 5.6 in 0.3 mM of NaCl (Na), procaine (P) or dibucaine (D) as analyzed with absorption spectrophotometry. The calcium values are related to the protein content (Lowry 1951) of the cell pellet. Column A gives the amount of calcium which was released to the extracellular medium after 30 min incubation in these solutions. All cells were washed in 1 mM NaCl pH 6.0 thereafter incubated in 1 mM $LaCl_3$ at pH 5.6 for another 30 min. Column B gives the amount of calcium removed from the cells during Na (dotted line) and La^{+++} (solid lines) treatment. After a final wash in NaCl (1 mM) the calcium which remained in the cell pellet after La^{+++} treatment is shown in column C. Each column in A represent 8 and in B and C 4 individual expts. S.E. are given as vertical bars in the columns.

cells than did NaCl or procaine (Fig. 8 column A). Of the total cell calcium the La^{++} sensitive portion which probably corresponds to Ca^{++} bound to the cell surface, was low in dibucaine treated cells (Fig. 8 column B). The calcium which remained was however not significantly lowered (column C) after the cells had been incubated in dibucaine or procaine solutions followed by $LaCl_3$. The loss of Ca^{++} from the cells could, however, not be directly responsible for the inhibitory effect of dibucaine since comparable or greater Ca^{++}-release occurred in media containing EGTA and these failed to inhibit pinocytosis (Table II).

In previous studies it has been shown that Ca inhibits channel formation when present in the period preceeding induction or during the pinocytosis cycle (Brandt and Freeman 1967, Cooper 1968, Josefsson 1968). Since local anesthetics might inhibit pinocytosis by a Ca

TABLE II. Comparison between Ca^{++} release from the cell and pinocytosis intensity. Amoebae cultured in Pringsheim solution were incubated for 30 min in different media (left column). All solutions were at pH 5.6 except the sodium salt of EGTA which was at pH 7.0. The release of calcium into the medium was measured by absorption spectrophotometry and the values are given as percent of the total calcium content of the sample ± S.E. After incubation in respective solutions, samples of the cells were tested for capacity to form channels in 100 mM tris-HCl at pH 7.0.

Extracellular medium	Ca^{++}-release/30 min (of total Ca)	Pinocytotic response to inductor (of max)
Distilled water	20 ± 0.4 (4)	33
Pringsheim	—	57
N Cl 0.3 mM	23 ± 2.3 (3)	100
Na_4EGTA 0.05 mM	36 ± 4.7 (4)	87
CFP	?6 ± 1.5 (8)	61
Na_4EGTA 0.1 mM	57 ± 1.6 (4)	31
Dibucaine 0.1 mM	30 ± 3.8 (4)	15
Na_4EGTA 0.3 mM	64 ± 1.2 (4)	8
Dibucaine 0.3 mM	31 ± 2.0 (12)	1



9. Effect of dibucaine pretreatment on calcium inhibition of potassium (A) and sodium induced pinocytosis (B). The Ca^{++} concentration of the inducing solution is given on the abscissa. The pinocytosis intensity is given on the ordinate as percentage of the pinocytosis elicited by the inducers 40 mM KCl pH 5.7 and 100 mM NaCl pH 7.0. Each experimental point indicates a single measurement on normal cells (circles) and cells pretreated for 30 min with 0.3 mM dibucaine pH 5.6 (squares).

10. Rate of oxygen uptake after induction of pinocytosis (curve A, B and D) and after the exchange of the medium (curve C). The measurements were made with cartesian micro-divers at 25°C. Pinocytosis induced by 100 mM tris-HCl pH 7.0. Oxygen uptake is given as percentage increase from the steady rate of O_2 uptake measured for a 10 min period before the introduction of the inducing solution at zero time. All curves start at ordinate zero. The manually controlled diver technique did not allow measurements to be made earlier than 10 min after the exchange of solution. Curve C indicates the normal transient increase in respiration following exchange (at zero time) of Pringsheim solution in the micro-diver. Curve B describes the respiratory increase in control cells during pinocytosis which follows upon exchange of 0.3 mM KCl for the inducing solution. Curve A represents conditions as for curve B with the exception that 0.3 mM dibucaine is present in the inducing solution. Curve D gives the increase of O_2 uptake during pinocytosis in cells pretreated for 30 min with 0.3 mM dibucaine. The conditions in A and D correspond to stimulated and inhibited pinocytosis respectively. Each experimental point is the mean of 5 experiments.

completing action, the interaction between the two types of pinocytosis blockers was examined. The interference of dibucaine with Ca^{++} inhibited pinocytosis was investigated in 40 mM KCl (pH 5.7) or 100 mM NaCl (pH 7.0) (Fig. 9 A and B). No additive effects of the two types of inhibitors were observed. On the contrary dibucaine pretreatment partly counteracted the Ca^{++} inhibition of pinocytosis. The intensity of pinocytosis induced by 40 mM KCl in the presence of 0.5–1 mM $CaCl_2$ did not differ significantly in control cells and cells pretreated with dibucaine. Using NaCl (100 mM, pH 5.7 and 7.0) or tris HCl (100 mM pH 7.0) as inducing salts prevention of pinocytosis by dibucaine was, however, only partially counteracted when Ca^{++} was added to the inducer.

Oxygen uptake after dibucaine treatment

During pinocytosis induced by several monovalent inorganic cations the oxygen uptake of the amoeba was increased (Hansson *et al.* 1968). This metabolic response to the pinocytosis inducer was reduced when Ca^{++} was added in concentrations which prevented pinocytosis. It was also found that mechanical manipulations of the cells as well as factors which stimulate channel formation increased the rate of respiration. The latter was measured with the

TABLE III Relative rate of oxygen in Pringsheim solution measured with a Clark electrode at 25°C. The cells were pre-incubated at room temperature with respective drug for 30 min. Readings were taken within 5 to 10 min after the cells were transferred to Pringsheim. The figures are percentages of the rates ±S.E. recorded in Pringsheim after incubation of aliquots of cells (about 30 min) in 0.3 mM NaCl. The number of experiments is given within brackets.

Pretreatment		Relative rate of O_2-uptake ±S.E.
Dibucaine	0.3 mM pH 5.6	69±6.6 (9)
Bupivacaine	0.3 mM pH 5.6	78±5.7 (7)
Procaine	0.3 mM pH 5.6	88±3.6 (7)
Vinblastine	0.3 mM pH 5.6	71±6.5 (7)
NaCl	0.3 mM pH 5.8	100 (control)

cartesian diver technique during pinocytosis in 100 mM tris HCl pH 7.0. The results are given in Fig. 10 where curve B represents the relative oxygen uptake during a normal cycle of pinocytosis. It is evident from the figure that the presence of dibucaine, which stimulates pinocytosis increased the rate of respiration (curve A), while pretreatment with the drug strongly depressed oxygen uptake (curve D). These findings with dibucaine are consistent with the view (Hansson *et al* 1968) that the degree of repiration is closely connected with channel formation. Pretreatment with the drug seemed to erase not only the effect of the inducer on respiration but also the normal respiratory increase after exchange of extracellular medium (curve C). From previous experiments with EGTA the rate of oxygen consumption was suggested to be determined by the Ca^{++} concentration in the cell membrane. Stimulated respiration was observed when the Ca^{++}-level was lowered from that prevailing during culture condition, while respiration was slowed down in cells both in Ca^{++} rich and in Ca^{++} deficient media. The different rates of respiration observed in the presence of dibucaine and after pretreatment with the drug might therefore reflect different degrees of Ca^{++} deficiency.

Measurements with a Clark electrode on suspensions of cells revealed that respiration of the cells in the culture medium was depressed after pretreatment with the drugs (Table III). The difference between the potent inhibitors of pinocytosis (dibucaine, vinblastine) and the indifferent or stimulating drugs (bupivacaine, procaine) was however small, so that interference with oxygen metabolism is not likely to determine the actions of these drugs on pinocytosis.

Effects of dibucaine on membrane potential and input resistance

When cells were transferred from culture fluid to a solution of 0.3 mM dibucaine, marginal changes of the membrane potential occurred during the subsequent 20 min (Fig. 11 A). As reported previously (Josefsson *et al* 1975) a prompt depolarization of the amoeba occurred when simple monovalent cations were added to the cells in concentrations which induce channel formation. Pretreatment with dibucaine, which completely prevents tris induced pinocytosis, did not, however, prevent depolarization due to the inducing cation (Table IV). Dibucaine also failed to reduce the increase in membrane conductance which followed the addition of these cations. This is illustrated in Fig. 11 B and C. In Fig. 11 B the input resistance of a cell was repeatedly measured during the pinocytosis period (in 20 mM NaCl) both in the culture fluid and during incubation with dibucaine. The de

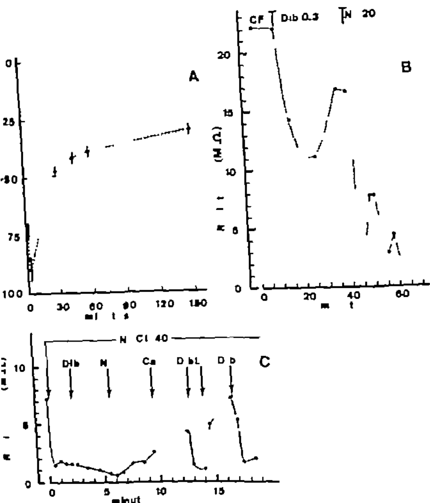


Fig. 11 A. Membrane potentials measured in cells incubated in 0.3 mM dibucaine pH 5.6 for varying lengths of time (abscissa). The values at zero time were obtained in Prescott's solution. Experimental points indicate the mean potential value ± S.E. obtained from 8–12 cells.

B. Input resistance of a cell in culture fluid (CF) followed by treatment with 0.3 mM dibucaine pH 5.6 and followed 30 min later by treatment with the inductor 20 mM NaCl pH 5.9. The ordinate gives resistance values expressed in MΩ obtained by the voltage clamp technique. The experiment simulates the procedure used for the study of pinocytosis inhibition by drugs.

C. Effect of dibucaine Ca^{++} and La^{+++} on input resistance of a cell immersed in 40 mM NaCl. In the course of the experiment the medium was exchanged (arrows in the figure) without altering the pH and NaCl concentration. The media used were introduced in the following order: 0.2 mM dibucaine, wash in 40 mM NaCl, 0.5 mM $CaCl_2$, 0.2 mM dibucaine, 0.5 mM $LaCl_3$, 0.2 mM dibucaine. Note that the presence of dibucaine coincides with low input resistances.

of membrane resistance upon incubation with the inducer was as prompt and as profound as in normal cells. In Table V the input resistance of normal and of dibucaine treated cells in three different solutions is summarized. In these media the anaesthetized cells had the lower resistances. Fig. 11 C demonstrates that the drug added to the inducer decreased the input

TABLE III Relative rate of oxygen in Pringsheim solution measured with a Clark electrode at 2[illegible]C. cells were pre-incubated at room temperature with respective drug for 30 min. Readings taken within 5 to 10 min after the cells were transferred to Pringsheim. The figures are perc… of the rates ±S.E. recorded in Pringsheim after incubation of aliquots of cells (about [illegible] in 0.3 mM NaCl. The number of experiments is given within brackets.

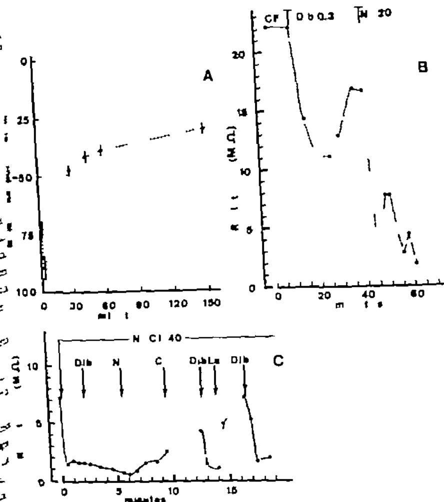
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g. 11 A. Membrane potentials measured in cells incubated in 0.3 mM dibucaine pH 5.6 for varying lengths of time (abscissa). The values at zero time were obtained in Prungsheim solution. Experimental points indicate the mean potential value ± S.E. obtained from 8–12 cells.

B. Input resistance of a cell in culture fluid (CF) followed by treatment with 0.3 mM dibucaine pH 5.6 and followed 30 min later by treatment with the inductor 20 mM NaCl pH 5.9. The ordinate gives resistance values expressed in MΩ obtained by the voltage clamp technique. The experiment simulates the procedure used for the study of pinocytosis inhibition by drugs.

C. Effect of dibucaine Ca^{++} and La^{+++} on input resistance of a cell immersed in 40 mM NaCl. In the course of the experiment the medium was exchanged (arrows in the figure) without altering the pH and NaCl concentration. The media used were introduced in the following order: 0.2 mM dibucaine, wash in 40 mM NaCl, 0.5 mM $CaCl_2$, 0.2 mM dibucaine, 0.5 mM $LaCl_3$, 0.4 mM dibucaine. Note that the presence of dibucaine coincides with low input resistance.

of membrane resistance upon incubation with the inducer was as prompt and as profound as in normal cells. In Table V the input resistance of normal and of dibucaine treated cells in three different solutions is summarized. In these media the anesthetized cells had the lower resistances. Fig. 11 C demonstrates that the drug added to the inducer decreased the input

TABLE IV The effect of dibucaine on membrane potential in *Amoeba proteus*. The cells w for 30 min either in Pringsheim pH 6.8 or 0.3 mM NaCl pH 5.8 or 0.3 mM dib Recording was made during a 30 min period following the incubation of the cell test solution. Tris 100 mM at pH 7.0 was used as pinocytosis inducer. Membrane in mV ± S.E. 15–20 measurements were made per experiment.

Pretreatment	Test solution	mV
Pringsheim	Dibucaine	−90.3 ± 6.1
Pringsheim	NaCl	−85.5 ± 6.4
Pringsheim	Tris 100 mM	−.20 ± 1.0
Dibucaine	Tris 100 mM	−19.6 ± 1.1
NaCl	Tris 100 mM	−17.7 ± 0.6

resistance even when the cells had been pretreated with Ca++ or La+++ contain It may be concluded that in contrast to Ca + dibucaine prevents pinocytosis creasing either the input resistance or the membrane potential of the cell.

Discussion

For a local anesthetic to prevent channel formation it was necessary that th contact with the cell, in a medium of low Ca + concentration for a certain p before addition of the inducing monovalent cation. This requirement and the at about pH 6 for the inhibitory effect might indicate that the drugs were taken by pinocytosis. Like Ca++ these drugs were weak inducers of pinocytosis in the unlike Ca++ they had no stabilizing effect on the electrical properties of the cell be correlated to the depression of pinocytosis. Yet the order of potency of inhibit pinocytosis was similar to the order of their anesthetic potency (Acevedo 1963 Truant and Takman 1965), and to the order in which the drugs inhibit cal to phospholipids (Blaustein and Goldman 1966 b) Furthermore the effect of ph and local anesthetics on pinocytosis was similar to the sequences given by Weinstein (1966) and Seeman (1966) for stabilizing erythrocytes against osmol This indicates that prevention of pinocytosis is another reflection of the membran effect of these drugs. Local anesthetic decreased respiration in the amoeba and dissociation of the bioelectrical effects of the inducer from the morphologica bolical phenomena of pinocytosis. This is in accord with their inhibition of caused by depolarization in conditions of excess extracellular K+ viz. contractu (Novotny *et al.* 1962), respiratory increase in muscle (Novotný and Vyskočil 196

TABLE V The effect of dibucaine pretreatment (0.3 mM for 30 min) on input resistance. MΩ ± S.E. The number of cells are within brackets. The pH of the test solutions

Test solution	Control cells	Dibucaine treated cells
NaCl 40 mM	.9 ± 0.21 (48)	4 ± 0.25 (4)
KCl 40 mM	0.7 ± 0.17 (8)	0.4 ± 0.11 (6)
KCl 10 mM	2.7 ± 0.55 (7)	2 ± 0.16 (10)

...ated secretion in the adrenal medulla (Jaanus *et al.* 1967). In these tissues Ca^{++} ex-... is also decreased by local anesthetic drugs (Lüttgau and Oetliker 1968, Rubin *et al.* ... Thus when the coupling between excitation and contraction or between stimulus ... ecretion are interrupted by local anesthetic drugs the binding and the movement of ... in membrane structures seems to be inhibited. It appears that a similar mechanism of ... might be relevant for the prevention of pinocytosis as reported here.

...e release of surface Ca^{++} from the amoebae in dibucaine solutions, the augmented ... of the drug in the presence of calcium binding agents and the weak inhibitory effect ... drug on Ca^{++}-depressed pinocytosis, points towards inhibition of pinocytosis due to ... deficiency. Surface Ca^{++} controls membrane permeability to ions (Brandt and Freeman ... Brandt and Hendil 1970, 1972, Josefsson *et al.* 1975). Drugs which release Ca^{++} would ... fore, as observed here with dibucaine, decrease transmembrane resistance and potential. ... strong effect of the drugs on sodium and tris induced pinocytosis compared to pino-...s induced by K ... might indicate, that surface calcium plays a more dominant role in ... cytosis induced by Na ... or tris ions. This is further supported by the observation that the ... ence of EGTA depresses tris (Josefsson 1968) and sodium induced pinocytosis more than ... ssium induced pinocytosis (see Fig. 7). Interference with the calcium metabolism in the ... eba might also be responsible for the depressed oxygen uptake, especially subsequent to ... application of the inductor since high concentrations of EGTA have been shown to ... rably decrease respiration in this cell (Hansson *et al.* 1968). The mere loss of Ca^{++} from ... cell surface in the presence of dibucaine could, however not explain the decreased channel ... nation. The drug might, in addition to its action as a simple counterion to fixed anions ... he surface membrane, penetrate into the lipids of the plasma membrane and displace ... from polar groups in this structure and even possibly from intracellular membranes. ... ocal anesthetics release membrane calcium from artificial lipid membranes and from ... ve and muscle (Hauser and Dawson 1968, Kuperman *et al.* 1968), and the cationic form ... dibucaine, when bound to the surface of the nerve membrane reduces the uptake of ... localcium (Suarez-Kurtz *et al.* 1970). Chlorpromazine and imipramine, both potent ... hibitors of pinocytosis, have also been shown to inhibit calcium transport in biological ... mbranes (Balzer *et al.* 1968). It might be that Ca^{++} bound in the plasma membrane of the ... oeba has to be released into the cytosol for the onset of channel formation. Replacement ... this Ca ... fraction by lipophilic organic bases in general might therefore directly inhibit ... factors which exchange with calcium at plasma membrane or otherwise cause calcium ... case form this site.

The effects of vinblastine on the amoeba were very similar to those of dibucaine. This drug ... no anesthetic action but like other lipid soluble cationic drugs it expands membranes ... d protects erythrocytes from osmotic hemolysis (Seeman *et al.* 1973). Like colchicine, ... hich did not acutely inhibit pinocytosis, vinblastine is known to affect microtubules (Mar... tz *et al.* 1969). It should be emphasized that only high concentrations of vinblastine in-...bited pinocytosis in the amoeba and that unspecific effects like those described by Seeman ... *et al.* (1973), common for lipid soluble organic bases, might therefore dominate. Recently ... son *et al.* (1970) found that vinblastine sulphate could precipitate a number of proteins ... erived from cell structures all of which were also precipitated by Ca^{++}. They concluded that

TABLE IV The effect of dibucaine on membrane potential in *Amoeba proteus*. The cells were pre… for 30 min either in Pringsheim pH 6.8 or 0.3 mM NaCl pH 5.8 or 0.3 mM dibucaine p… Recording was made during a 20 min period following the incubation of the cells in … test solution. Tris 100 mM at pH 7.0 was used as pinocytosis inducer. Membrane potentia… in mV ± S.E. 15–20 measurements were made per experiment.

Pretreatment	Test solution	mV
Pringsheim	Dibucaine	−90.3 ± 6.1
Pringsheim	NaCl	−85.5 ± 6.4
Pringsheim	Tris 100 mM	−22.0 ± 1.0
Dibucaine	Tris 100 mM	−19.6 ± 1.1
NaCl	Tris 100 mM	−17.7 ± 0.6

resistance even when the cells had been pretreated with Ca^{++} or La^{+++} containing solut… It may be concluded that in contrast to Ca^{++} dibucaine prevents pinocytosis without … creasing either the input resistance or the membrane potential of the cell.

Discussion

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TABLE V The effect of dibucaine pretreatment (0.3 mM, t = 30 min) on input resistance. The value… MΩ ± S.E. The number of cells are within brackets. The pH of the test solutions … 5.8.

Test solution	Control cells	Dibucaine treated cells
NaCl 40 mM	2.9 ± 0.21 (48)	2.4 ± 0.[illegible]5 (24)
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O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall, Protein measurement with the Folin ...ol reagent. *J. Biol. Chem.* 1951 *193*, 265–275.

...ai, H. C. and H. Oetliker, The action of caffeine on the activation of the contractile mechanism ...iated muscle fibres. *J. Physiol.* (Lond.) 1968. *194* 51 74.

...vez, R., M. Ventilla and M. Shelanski, Vinblastine-induced precipitation of microtubule protein. ...nce 1969. *165* 498–499.

...all, J. M. and V. T. Nachmias, Cell surface and pinocytosis. *J. Histochem. Cytochem.* 1965. *13*, 104.

...ivy, I. and F. Vyskočil, Possible role of Ca ions in the resting metabolism of frog sartorius muscle ...ng potassium depolarization. *J. cell. comp. Physiol.* 1966. *67* 159–168.

...ivy, L., F. Vyskočil, L. Vyklický and R. Beránek, Potassium and caffeine induced increase of ...gen consumption in frog muscle and its inhibition by drugs. *Physiol. Bohemoslov.* 1962. *11* 277–284.

...i, R. P., M. R. Feinstein, S. D. Jaanus and M. Paimre, Inhibition of catecholamine secretion and ...ion exchange in perfused cat adrenal glands by tetracaine and magnesium. *J. Pharmacol. exp. Ther.* ...7 *155* 463–471

...arzenbach, G. Die komplexometrische Titration. 4.e Auflage, Stuttgart 1960.

...n, P. Erythrocyte membrane stabilization by local anesthetics and tranquilizers. *Biochem. Pharmacol.* ...6 *15*. 1753–1766.

...n, P., M. Chan-Wong and S. Moyyen, Membrane expansion by vinblastine and strychnine. *Nature* *Biol.* 1973, *241* 22.

...n, P. and I. Weinstein, Erythrocyte membrane stabilization by tranquilizers and antihistamines. ...chem. *Pharmacol.* 1966. *15* 1737–1752.

...er-Kuntz, G., C. P. Bianchi and P. Krupp. Effect of local anesthetics on radiocalcium binding in ...ve. *Europ. J. Pharmacol* 1970. *10*, 91 100.

... A. P. and B. Takman, Local Anesthetics. In *Drill' Pharmacology in Medicine*, 3rd Edition, ed. ...R. Di Palma, New York, McGraw-Hill, 1965. 148–156.

...on, L., J. Bryan, A. Ruby and D. Mazia, Precipitation of proteins by vinblastine and calcium ions. ...c. nat. Acad. Sci. (Wash.) 1970. *46*. 807–814.

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This work was supported by the Swedish Cancer Society, project number 167-K69-02X and the Medical Faculty of the University of Lund, Sweden.

The following firms graciously donated drug samples: AB Leo, Helsingborg, Sweden (chlorpromazine-HCl and prochlorperazine methanesulphonate), AB Astra, Södertälje, Sweden (lidocaine-HCl and 572), AB Bofors, Mölndal, Sweden (mepivacaine-HCl, bupivacaine (Marcain®)), E. Lilly, Indianapolis, Inc., USA (vinblastine sulphate), Sandoz, Basle, Switzerland (thioridazine-HCl), AB Recip, Stockholm, Sweden (promethazine-HCl and N-hydroxyethylpromethazine Cl (Aprobit®)), Ciba, Basle, Switzerland (dibucaine).

References

ACEVES, J. and X. MACHNE, The action of calcium and of local anesthetics on nerve cells, and their action during excitation. *J. Pharmacol. exp. Ther.* 1963. *140*. 138–148.

BALZER, H. and M. MAKINOSE, The inhibition of the sarcoplasmic calcium pump by drugs which react with its lipid component: reserpine, prenylamine, chlorpromazine, imipramine. *N.-Schmiedeberg Arch. exp. Path. Pharmak.* 1968. *259*. 151

BLAUSTEIN, M. P. and D. E. GOLDMAN, Competitive action of calcium and procaine on lobster axon. *J. gen. Physiol.* 1966 a. *49*. 1043–1063.

BLAUSTEIN, M. P. and D. E. GOLDMAN, Action of anionic and cationic nerve-blocking agents. Experiment and interpretation. *Science* 1966 b. *153*. 429–432.

BRANDT, P. W. and A. R. FREEMAN, Plasma membrane. Substructural changes correlated with electrical resistance and pinocytosis. *Science* 1967. *155*. 582–585.

BRANDT, P. W. and K. B. HENDIL, Properties of the plasma membrane of amoebae. In. Bittar, E. E. *Advance in Exp. Medicine and Biology* 1970. *7*. 323–335

BRANDT, P. W. and K. B. HENDIL, Plasma membrane permeability and pinocytosis in *Chaos Chaos*. *C. R. Lab. Carlsberg* 1972. *38*. 423–443

COOPER, B. A., Quantitative studies of pinocytosis induced in *Amoeba proteus* by simple cations. *C. R. Lab. Carlsberg* 1968. *36*. 385–403

FEINSTEIN, M. B., Reaction of local anesthetics with phospholipids. A possible chemical basis for anesthesia. *J. gen. Physiol.* 1964. *48*. 357–374.

HANSSON, E. E., G. JOHANSSON and J. O. JOSEFSSON, Oxygen uptake during induced pinocytosis in *Amoeba proteus*. *Acta physiol. scand.* 1968. *73*. 491–500.

HAUSER, H. and R. M. C. DAWSON, The displacement of calcium ions from phospholipid monolayers by pharmacologically active and other organic bases. *Biochem. J.* 1968. *109*. 909–916.

HENDIL, K. B. Ion exchange properties of the glycocalyx of the amoeba *Chaos Chaos* and its relation to pinocytosis. *C. R. Lab. Carlsberg* 1971. *38*. 187–211

JAANUS, S. D., E. MIELE and R. P. RUBIN. The analysis of the inhibitory effect of local anesthetics and propranolol on adrenomedullary secretion evoked by calcium or acetylcholine. *Brit. J. Pharmacol.* 1967. *31*. 319–330

JOHNSON, C. L. and A. SCHWARTZ, Some effects of local anesthetics on isolated mitochondria. *J. Pharmacol. exp. Ther.* 1969. *67*. 365–373

JOSEFSSON, J.-O. Induction and inhibition of pinocytosis in *Amoeba proteus*. *Acta physiol. scand.* 1968. *73*. 481–490.

JOSEFSSON, J.-O., N.-G. HOLMER and E. E. HANSSON. Membrane potential and conductance during pinocytosis induced in *Amoeba proteus* with alkali metal ions. *Acta physiol. scand.* 1975. *94*. 278–288

KUPERMAN, A. S., B. ALTURA and J. CHEZAR, Action of procain on calcium efflux from frog nerve and muscle. *Nature* (Lond.) 1968. *217*. 673–675.

…y, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall, Protein measurement …ith the Folin …ol reagent. *J. Biol. Chem.* 1951 *193*, 265–275.

…al, H. C. and H. Oetliker, The action of caffeine on the activation of the contractile mechanism …ed muscle fibers. *J. Physiol.* (Lond.) 1968, *194* 51–74.

…tz, R., M. Ventilla and M. Shelanski, Vinblastine-induced precipitation of microtubule protein. …ce 1969, *165*, 498–499.

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…arzenbach, G. Die komplexometrische Titration. 4. Auflage, Stuttgart 1960.

…n, P. Erythrocyte membrane stabilization by local anesthetics and tranquilizers. *Biochem. Pharmacol.* …5 *15*, 1753–1766.

…n, P., M. Chan-Wong and S. Mottrem, Membrane expansion by vinblastine and strychnine. *Nature* *Biol.* 1971, *241* 22.

…n, P. and I. Weinstein, Erythrocyte membrane stabilization by tranquilizers and antihistamines. …chem. Pharmacol. 1966, *15* 1737–1752.

…-Kurtz, G., C. P. Bianchi and P. Krupp. Effect of local anesthetics on radiocalcium binding in … *Europ. J. Pharmacol.* 1970, *10*, 91–100.

…t, A. P. and B. Takman, Local Anesthetics. I. *Drill' Pharmacology in Medicine* 3rd Edition, ed. … Di Palma, New York, McGraw-Hill, 1965, 148–156.

…n, L., J. Bryan, A. Ruby and D. Mazia, Precipitation of proteins by vinblastine and calcium ions. … nat. Acad. Sci. (Wash.) 1970, *66*, 807–814.

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References

ACEVES, J. and X. MACHNE, The action of calcium and of local anesthetics on nerve cells, and th action during excitation. *J. Pharmacol exp. Ther.* 1963. *140*. 138–148.

BALZER, H. and M. MAKINOSE, The inhibition of the sarcoplasmic calcium pump produced b which react with its lipid component: reserpine, prenylamine, chlorpromazine, imipramine. *Schmiedebergs Arch. exp. Path. Pharmak.* 1968. *259*. 151.

BLAUSTEIN, M. P. and D. E. GOLDMAN, Competitive action of calcium and procaine on lobst *J. gen. Physiol.* 1966 a. *49*. 1043–1063.

BLAUSTEIN, M. P. and D. E. GOLDMAN, Action of anionic and cationic nerve-blocking agents: Ex and interpretation. *Science* 1966 b. *153*. 429–432.

BRANDT, P. W. and A. R. FREEMAN, Plasma membrane: Substructural changes correlated with (resistance and pinocytosis. *Science* 1967. *155*. 582–585.

BRANDT, P. W. and K. B. HENDIL, Properties of the plasma membrane of amoeba. In: Biol.) *Advances in Exp. Medicine and Biology* 1970. *7*. 323–335.

BRANDT, P. W. and K. B. HENDIL, Plasma membrane permeability and pinocytosis in *Chaos* (*R. Lab. Carlsberg* 1972. *38*. 423–443.

COOPER, B. A. Quantitative studies of pinocytosis induced in *Amoeba proteus* by simple cation *Lab. Carlsberg* 1968. *36*. 385–403.

FEINSTEIN, M. B. Reaction of local anesthetics with phospholipids. A possible chemical basis for an *J. gen. Physiol.* 1964. *48*. 357–374.

HANSSON, S. E., G. JOHANSSON and J. O. JOSEFSSON, Oxygen uptake during induced pinocytosis in *proteus. Acta physiol. scand.* 1968. *73*. 491–500.

HAUSER, H. and R. M. C. DAWSON, The displacement of calcium ions from phospholipid monola pharmacologically active and other organic bases. *Biochem. J.* 1968. *109*. 909–916.

HENDIL, K. B., Ion exchange properties of the glycocalyx of the amoeba *Chaos Chaos* and its rel pinocytosis. *C. R. Lab. Carlsberg* 1971. *38*. 187–211.

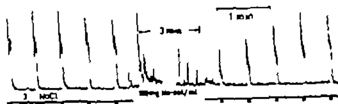
JAANUS, S. D., E. MIELE and R. P. RUBIN, The analysis of the inhibitory effect of local anesthe propranolol on adrenomedullary secretion evoked by calcium or acetylcholine. *Brit. J. Ph* 1967. *31*. 319–330.

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JOSEFSSON, J.-O., N.-G. HOLMER and S. E. HANSSON, Membrane potential and conductance durin cytosis induced in *Amoeba proteus* with alkali metal ions. *Acta physiol. scand.* 1975. *94*. 278–28

KUPERMAN, A. S., B. ALTURA and J. CHEZAR, Action of procain on calcium efflux from frog ne muscle. *Nature* (Lond.) 1968. *217*. 673–675.



The record shows the response to 0.3 M NaCl before and after the application of sodium salicylate, mg/ml, on the tongue for 3 min. The record demonstrates that the taste response was depressed after salicylate.

Methods

Adult male Sprague-Dawley rats were used. They were anaesthetized with Hypnorm (Leo), which is a combination of a narcotic analgesic, 0.2 mg/ml of fentanyl, and a tranquilliser 10 mg/ml of fluanisone, originally adapted for veterinary use. The initial dose of 0.5 ml/kg b. w. was injected i.m. The anaesthesia was maintained with a barbiturate, Mebumal containing 60 mg sodium pentobarbital/ml. The right chorda tympani proper nerve, which is a branch from the facial nerve to a trigeminal branch, the lingual proper nerve, was dissected. It mediates taste sensations from the anterior part of the tongue. The activity of the nerve was recorded under mineral oil, amplified and displayed on an oscilloscope. It was then recorded on a recorder, Varian, as summated trace. The tongue was stimulated with solutions of 0.3 M NaCl, 0.3 or 0.5 M sucrose, 0.02 M acetic acid and 0.02 quinine hydrochloride for about 3 s, preceded and followed by tap water at the same temperature. The device used to apply the taste stimuli has already been described in detail (Andersson *et al.* 1971). The taste solutions are housed in a temperature-controlled water bath. The flow of these solutions was regulated through magnetic valves operated by electronic circuits. The timing and sequence of the stimuli could be programmed. This technique allows reproducible, well-controlled stimulation of the taste buds.

Aspirin (acetyl salicylic acid) and sodium salicylate have been tested. When aspirin was used, 72 mg aspirin and 52 mg tris (hydroxymethyl) aminomethane were made up in 1 ml distilled water. The solution was titrated to pH 7.4. The sodium salicylate which was used locally was dissolved in water 50, 75, 100, 200 and 500 mg/ml. For intravascular infusion it was dissolved in Haemaccel (a plasma substitute) or in Ringer solution. Three different concentrations were used, 10, 25 and 100 mg/ml solvent. Indomethacin was also used. Before we had access to a water-soluble substance, through the courtesy of Dumex, it was dissolved in dimethyl sulfoxide or alcohol or in a mixture of these two. When water-soluble indomethacin was available, it was dissolved in concentrations of up to 30 mg/ml water or Ringer's solution.

The systemic blood pressure was recorded in one femoral artery. For direct infusion into the tongue of drugs, the right external maxillary artery was catheterized. The tip of the catheter was placed close and peripheral to the branching of the lingual artery. This left the blood supply to the tongue undisturbed and allowed direct infusion of substances into the vascular bed of the tongue. The infusion was made with a infusion pump (Holter RD 074) the speed of which was monitored on the simultaneously recorded taste response. With three-way valves in the loop, different kinds of solutions could be selected. To prevent coagulation in the catheter we added 100 I.U. of heparin/ml solution.

Results

The effect of salicylates on the taste response has been studied with 3 different methods of application. Fig. 1 shows an example of the effect of topical application to the tongue. The responses to repeated stimulation with 0.3 M NaCl were recorded before and after the application of 100 mg sodium salicylate/ml for 3 min. Fig. 1 shows that after the application the response to the salt solution was depressed. It can be noticed that the salicylate solution in itself increased the neural activity of which only the initial and last parts have been included in Fig. 1. These experiments were also repeated with solution of aspirin which was

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Depression of Taste Responses by Local or Intravascular Administration of Salicylates in the Rat

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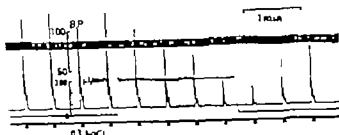
Abstract

HELLEKANT G and V GOPAL. *Depression of taste responses by local or intravascular administration of salicylates in the rat* Acta physiol. scand. 1975 95 286–292.

The effects of aspirin and sodium salicylate on the taste response in the chorda tympani proper nerve have been studied during stimulation of the taste receptors in rats with salty, acid, and bitter-tasting solutions. Three methods of administering the drugs were used locally on the tongue, intravenously into the femoral vein and intraarterially close to the branching of the lingual artery. It was observed that salicylates, given in any of these three ways, depressed the response to taste stimuli. It is discussed in relation to present views on the action of salicylates.

In an earlier series of experiments in which we studied the relationship between taste activity and blood flow through the tongue in the rat we had problems with thrombocyte aggregation (Hellekant 1971). We were advised to give salicylate to prevent this. However, when we then tried to record a response to taste stimulation of the tongue we failed to record any response. We soon suspected that this failure was related to the salicylate injected. Salicylates are capable of alleviating certain types of pain (Goodman and Gilman 1970) Some of the pain relief seems to be the result of an effect on peripheral receptors (Collier 1969). Thus it had been reported that salicylates in dog block apparently pain stimulation of certain visceral chemoreceptors evoked by intraarterially injected bradykinin (Lim *et al.* 1964). Further Scott (1968) concluded that topical application of aspirin to the dentinal receptors in cats promptly inhibits both the steady-state discharge and the response to brief heat stimulation. However these earlier reports describe effects of salicylates on receptors responding to noxious stimulation No report has suggested an effect of salicylate on receptors responding to non-painful stimuli such as weak salt solutions on the tongue. Our unwanted finding was therefore surprising. It seemed worth while to explore this unexpected effect of salicylates in more detail. A short preliminary report has already been made (Hellekant 1971)

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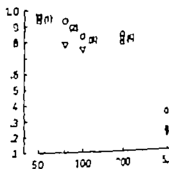


4. The effect of infusing sodium salicylate into the tongue on the taste response to 0.3 M NaCl as ...ted. The top trace illustrates the systemic blood pressure, the middle one the taste response and the ...st one monitors the rate of infusion. The record shows that after about 1.5 mg there was definite ...ution of the taste response, while the effect on the blood pressure was small.

However, as shown by Fig. 3, a fairly high dose of salicylate had to be given when the ...stance was infused systemically. General effects of salicylate like transcapillary bleeding, ...nges in systemic blood pressure, etc. were frequently observed. In order to avoid these ...ects and to study the effect of intravascularly administered salicylates further we infused ... substances via the external carotid artery directly into the tongue, as described in ...thods. In this case a much lower dose gave an effect. Fig. 4 shows an example of this. ... solution containing 10 mg sodium salicylate/ml solution was infused at a rate of 300 .../min. After about 1.5 mg there was a definite diminution of the taste response. Provided ...at the dose was not too large, the taste sensitivity usually then returned to the previous ...el when the infusion stopped. The cumulative effect observed during general systemic ...ministration was less evident with this technique, but could be observed. The top trace ... Fig. 4 shows the systemic blood pressure recorded in one femoral artery. This dose of ...icylate did not effect it.

An effect of salicylates on peripheral receptors has been demonstrated by other authors ...am 1966, Scott 1968). Since it is now generally thought (*cf.* Vane 1973) that salicylates ...ert their effects via an inhibition of prostaglandins, we decided to study the effect of a ...bstance which has a similar analgetic effect as salicylates but which is a stronger inhibitor ...of prostaglandins. Indomethacin was chosen because it has been described as being at least ...0 times more potent than aspirin in its inhibition of prostaglandins. We have studied the ...fect of indomethacin on the taste response after topical as well as intravascular application. ...13 rats we tried infusion of solutions containing ..., 5, 10 and 50 mg indomethacin/ml at ...fusion rates varying from 100 to 300 µl/min. We found that it was necessary to infuse ...about 2 mg at an infusion rate of not less than 1 mg/min to cause a depression of the taste ...sponses. An example of a recording obtained during infusion with 5 mg indomethacin/ml ...olution at a rate of 300 µl/min into the tongue is shown in Fig. 5. This record shows that ...no effect on the taste sensitivity was elicited although about 1.7 mg of indomethacin was ...infused. Further in 6 rats we applied indomethacin on the tongue in a concentration of 50 ...mg/ml. In 2 rats we used water-soluble indomethacin but observed no effects on the taste ...response. In 4 rats we used the usual commercially available substance which was dissolved ...in alcohol or dimethyl sulfoxide. In no case did we record an effect on the taste response.

Fig. 2. The ratios between the responses before and after sodium salicylate for 3 min on the tongue have been plotted against the concentration of salicylate used. The number of animals in each set of data is indicated by the figures in brackets. 3 different taste stimuli were used, 0.3 M NaCl (□), 0.02 M citric acid (O) and 0.02 M quinine hydrochloride (∇). The diagram indicates that a definite suppression of the taste sensitivity occurred at 100 mg salicylate/ml.



rinsed over the tongue. The taste response was depressed in a similar way as shown 1 In 10 rats we studied the dose-effect relationship by applying sodium salicylate different concentrations on the tongue for 3 min. The results are illustrated in Fig. 2, the relationship between the responses before and after the salicylate is expressed as The peak responses have been used. The first 4–5 responses of each stimulus a salicylate have been used. Fig. 2 shows that the responses to all stimuli used were Since we already knew that salicylate in higher concentration depresses the taste re our interest focused on the concentration of salicylate that was necessary to cause a sion One hundred mg/ml was considered to be a "critical" concentration. There applied this concentration to all animals except the first one. From Fig. 2 it can be se that the concentration that definitely causes a depression is somewhere between 100 mg. A significance test, statistics for two means, using the ratios between the re before and after salicylate, gave a significant effect of 100 mg salicylate/ml solution (p on the tongue. The depression caused by 500 mg/ml was strong and lasted for mo 30 min in spite of a continuous rinse of the tongue with water

It seemed of interest to see if salicylates applied from within the taste buds wou an effect on the taste receptors. Fig. 3 shows an example of such an experiment. In we infused the drug through one femoral vein. The animal received in total 200 mg as b.wt. In Fig. 3 the first bottom mark indicates the onset of infusion and the second termination. In this rat after about 100 mg/kg b wt. a significant decrease of the t sponse could be recorded. A slight trace of hypersensitivity is also seen before the d

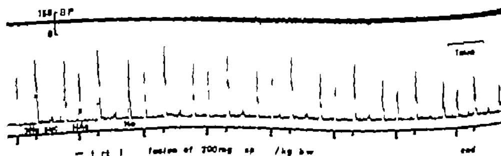


Fig. 3. The effect on the taste response of i infusion of 200 mg aspirin/kg b. t. is shown. Th shows a decrease in taste sensitivity as the result of aspirin.

y are then thought to work near the spot where they were released, giving rise to noxious -tions. Thus salicylates are supposed to depress *only the* responses from receptors ted by noxious stimulation and not by "normal" stimulation.

his view makes our finding that salicylates diminish the *sensitivity of the taste buds to* s stimulation rather unexpected.

here appears to be two possible explanations. Either prostaglandins are in some way ved in the generation of the taste responses from the taste buds, or the effects of sali- es on these receptors differ from what at present is regarded as their mode of action receptors (Collier 1971). For this reason we tested Indomethazin, known to be a power inhibitor of the release or synthesis of prostaglandins. It is at least 10 times more potent a aspirin. Thus Vane (1973) reports data which suggest that it is 17 to 2140 times more ent as an inhibitor of prostaglandin synthesis. Our results show that Indomethazin was *e to suppress the taste sensitivity but* the concentration needed was not lower than that salicylates. It is possible that a difference in ability to penetrate the tissue may have yed a role, but the fact that when it did have an effect, the latency was about the same for salicylate makes it more probable that prostaglandins have nothing to do with the eration of the taste response. This conclusion is also supported by a brief experiment h prostaglandin $F_{2\alpha}$ in which 33 μg and 63 μg were infused into the tongue over 2 min two separate experiments. A strong effect on the blood pressure was observed but no ect on the taste response. Prostaglandin E_1 would have been a better choice, because it s been used as a pain-producing agent, but was not available. However from the data scribed we conclude that the action of salicylates on the taste receptors differ from what at present regarded as its mode of action on receptors (Collier 1971 Vane 1973).

There are a number of effects of salicylates which can be regarded as possible explana- ns of our observations. Salicylates have general effects on metabolic processes (Good- an and Gilman 1970). They uncouple oxidative phosphorylation (Smith and Smith 1966), a result of which, number of adenosine triphosphate dependent reactions are inhibited. his gives rise to an increased demand for oxygen, as a result of the enhanced oxidation mpensating for the relative inefficiency of the phosphorylating mechanisms. Since the ste cells have a high demand for oxygen (Hellekant 1971) *the decrease in sensitivity de-* ribed might have been the result of an inability to satisfy this increased demand. The phase) hypersensitivity *during the initial stage of salicylate administration* supports this, be use it is general feature of a receptor that its threshold diminished initially when its bility to maintain its membrane potential deteriorates, as a result of hypoxia, for example.

In summary this study shows that salicylates after topical application or intravascular dministration to the tongue diminish and finally abolish the response of the taste receptors) taste stimulation. Experiments with indomethazin, known to be a more powerful prosta- landin inhibitor indicate that this effect of salicylate on the taste receptors is probably nrelated to its effect on prostaglandins. This effect has been regarded as the mode of ction of salicylate when suppressing the response from other peripheral receptors.

This study was supported by grants from Maktdrycknäringsfonden, Knut och Alice Wallenbergs Stiftelse, Magnus Bergvalls Stiftelse and the Swedish Medical Research Council grant No 2447
The supply of water-soluble indomethazin from Dumex Läkemedel AB is gratefully acknowledged.

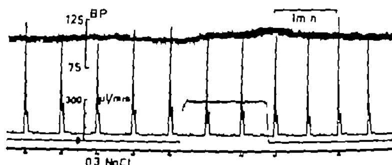


Fig. 5. Indomethazin, 5 mg/ml, was infused into the tongue at the same rate as in Fig. 4. The tota-
given was about 1 7 mg. No depression of the taste responses was observed.

Since the concentrations of indomethazin we used without obtaining any effect were ab
the same as those of salicylate, and since it is claimed that indomethazin is at least a 10 ti
(Vane 1973) more efficient inhibitor of prostaglandin than aspirin, we conclude that t
effects of salicylate described here on the taste sensitivity were not the result of its eff
on prostaglandins.

Discussion

This discussion will first focus on the dose of salicylates used and the possibility that t
depression of the taste sensitivity observed might have been the result of a general int
cation.

Salicylates were formerly regarded as harmless drugs. This opinion has changed l
salicylates are still widely used. In man the therapeutic maximum dose seems to be l
mg/kg b.wt. and day (Goodman and Gilman 1970). In rat the LD_{50} is 500 mg/kg intrap
toneally (Ichniowski and Hueper 1946) and 125 mg/kg blocks bradykinin-induced noci
tion (Blane 1967) From these figures it seems to us that with the doses we used for the
administration a general effect can be suspected as underlying the depression of taste s
sitivity described here. However this would not explain the depression observed after lo
application to the tongue, when only a few mg could have entered the body

The strong effect of local application is somewhat surprising, because the taste buds
known to withstand all kinds of toxic substances. The number of substances which penet
the epithelium of the tongue is limited (Mistretta 1971) The present results indicate a ra
absorption of salicylates through the epithelium of the tongue similar to the rapid abso
tion through the intact skin (Goodman and Gilman 1970). Judged by the almost immed
discharge from the taste cells on the topical application of salicylates and the slower ef
of infusion, it appears that the taste cells are the main target for salicylate and not the ne
fibres. This is supported by the findings of Lim *et al* (1964) and Scott (1968) that the i
pulse conduction in peripheral nerve is not affected by aspirin

Previous studies (Lim 1966 Scott 1968) have demonstrated an effect of salicylates on
response to noxious stimulation. The present opinion seems to be (*cf* Collier 1971 Va
1973) that salicylates exert this effect via a blocking of the synthesis or release of pro
glandins, which are known to increase locally as the result of in vivo inflammation

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Kinetics of the Glomerular Ultrafiltration in the Rat Kidney An Experimental Study

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Abstract

[illegible]llskog, Ö., L. O. Lindbom, H. R. Ulfendahl and M. Wolgast *Kinetics of the glomerular ultrafiltration in the rat kidney An experimental study* Acta physiol. scand. 1975 95 293–300.

[illegible] quantitative relation between the driving forces over the glomerular membrane and the glomerular [illegible] flow, on the one hand, and the single glomerular filtration rate (SNGFR), on the other [illegible] still [illegible]. Micropuncture measurements on Sprague-Dawley rats made it possible to calculate the net [illegible] force over the glomerular membrane. The single glomerular plasma flow was determined from [illegible] GFR and the single nephron filtration fraction (SNFF). The effective plasma flow was measured with [illegible] H for total kidney and for superficial nephrons. The mean glomerular capillary pressure [illegible] found to [illegible] 6 mm Hg. The results indicate [illegible] net driving force of about 13 mm Hg at the distal end of the glomerular capillary. SNGFR [illegible] found to be 14 1 nl/min 100 g. SNFF amounted to about 0.27 The filtration fractions determined with the PAH method were in the same range. The results indicate [illegible] filtration equilibrium, in contrast to those of Brenner *et al.* from measurements on [illegible] mutant Wistar rat strain. The [illegible] fractions seemed to be the same in all glomerular populations. It is clear that the SNGFR is [illegible] dependent. Our earlier findings of [illegible] nonautoregulation of the blood flow through the outer glomeruli were also confirmed.

[illegible]hile the sieving properties of the glomerular membrane have been extensively studied, [illegible]formation about the hydrostatic and oncotic forces operative over the glomerular membrane and about the glomerular plasma flow and the filtration fraction are still scanty [illegible] With the introduction of the servo-nulling micropipette technique by Wiederhielm *et al.* [illegible]964) it became possible to record the hydrostatic pressures within the superficial renal [illegible]sculature. The glomeruli are usually not located in the superficial layer of the kidney [illegible] mutant Wistar strain with superficial glomeruli Brenner *et al.* (1971 [illegible]) were able to [illegible]erform direct punctures of the glomerular capillaries. They found a glomerular capillary [illegible]ressure of about 45 mm Hg, [illegible] pressure which has since been confirmed by several workers [illegible] Brenner *et al.* 1972, Robertson *et al.* 1972, Blantz *et al.* 1972). This pressure is considerably [illegible]wer than that obtained by the indirect stop-flow technique on the Sprague-Dawley rat [illegible] Gertz *et al.* 1966, 1969 Koch *et al.* 1967 Krause *et al.* 1967 Andreucci *et al.* 1971). Brenner

References

ANDERSSON, I G HELLEKANT R. LARSSON, C. MOLANDER and L. STRÖM, A system for [illegible] matically controlled gustatory stimulation. *Med. Biol. Engin.* 1971 *9*, 715–717

BLANE, G Blockade of bradykinin induced nociception in the rat as a test for analgesic drugs [illegible] reference to morphine antagonists. *J Pharm. Pharmac* 1967 *19* 367–373.

COLLIER, H O J Prostaglandins and aspirin. *Nature* (Lond.) 1971 *232* 17–19.

GOODMAN, L. S and A. GILMAN, *The pharmacological basis of therapeutics*. The Macmillan Co 1970 IV ed. 1794 p.

HELLEKANT G The effect of changes in the blood flow to the tongue on the chorda tympani [illegible] rat. *Acta physiol. scand.* 1971 *82*, 145–153.

HELLEKANT G., The effect of acetylsalicylic acid on the taste response of the rat. *Proc. int. Union Physiol.* 1971 *9* 715

ICHNIOWSKI C. T and W C. HUEPER, Pharmacological and toxicological studies on salicylamide. *J. Am. pharm. Ass.* 1946. *35* 2-5–230.

LIM, R. K. S., *Salicylate analgesia*. In the salicylates, a critical bibliographic review 1966. John Wiley & Sons, Inc. New York.

LIM, R. K. S., F GUZMAN, D W RODGERS, K. GOTO, C. BRAUN, G D. DICKERSON and R. J. E. Site of action of narcotic and non-narcotic analgesics determined by blocking bradykinin-evoked visceral pain. *Arch int Pharmacodyn.* 1964. *152*, 25–58.

MISTRETTA, C. M Permeability of tongue epithelium and its relation to taste. *Amer J. Physiol.* 220, 1162–1167

SCOTT D Jr Aspirin action on receptor in the tooth. *Science* 1968. *161* 180–181

SMITH, M J and P K. SMITH, *The salicylates*: a critical bibliographic review John Wiley & Sons 1966. New Yo k.

VANE, J R., Inhibition of prostaglandin biosynthesis as the mechanism of action of aspirin-like drugs. *Biochem.* 1973 *9* 395–411

the *second series* the total and single nephron GFR as well as the total and single nephron PAH ance were measured in addition to pressure measurements in the superficial vascular and tubular teres. The single nephron PAH clearance was measured by quantitative sampling in an oil-blocked tubular segment. These segments were identified by injections of Lissamine green through the pressure ording pipette into proximal tubular segments. This pipette remained in position under the sampling to control an adequate suction. The volume of the sample was measured from the length in constant capillaries (Microcaps, 0.5 µl, Drummond).

The experiments were performed during normo- and hypotensive conditions. The latter was induced by a clamp between the right and left renal arteries.

The radioactivity of the 125-I PAH and the 3-H Inulin used was measured in a liquid scintillation counter (Nuclear LS-250).

In the *third series* the filtration fraction of single nephrons was determined. 125-I labelled albumin was used and the fraction was calculated from the concentration of radioactivity in plasma samples from the welling points as related to the concentration of the activity in systemic plasma. The sampling of blood from the welling points proved to be very difficult in the adult, full-grown Sprague-Dawley rats. To prevent clotting, all animals were heparinized with an intravenous injection of 2 000 I.U. heparin /kg b.wt. All samplings were performed within one hour after the injection of the heparin. The tip diameter of the cannulae used was about 12 µm and the cannulae were filled with coloured mineral oil except for the very tip, which was filled with coloured heparin-saline solution. All punctures were made at a relatively small angle, either directly into the welling point or into a first order branch with the tip pushed forward into the welling point. The coloured heparin-saline solution was then injected by the syringe to make sure that the puncture was purely intravascular – all punctures at which the dye leaked out into the tubules or the interstitium were discarded. The blood was collected by spontaneous flow or by sampling against a slight counter pressure. By changing the counter pressure with the syringe the operator regulated the speed of the streaming of the red cells in the tip of the cannula to be fairly constant. In some control experiments the pressure was measured just proximal to the sampling pipette and showed no divergence from that in intact welling points. Smaller vessels or the first order branches not rarely showed signs of vasoconstriction with subsequent reduction in the sampling rate, in which case the samples were discarded. After completion of the sampling the cannulae were sealed with the oil superfusing the kidneys. The samples were then put into microcapillary tubes (Microcaps, 0.5 µl Drummond), sealed at one end by burning, and centrifuged for 2 min at 8 000 g. The volume of the plasma and red cells was determined, thereafter the samples were analysed in a gamma-spectrometer. The hematocrit of the welling point blood as well as of the systemic blood was also calculated. These variables were not used in the determination of the filtration fraction, though essentially the same results would have been obtained.

In the *fourth series of experiments the effective driving force over the glomerular membrane was changed* by applying a counter pressure on the tubular side of the membrane. This was achieved by continuous injection of oil into *late proximal segment until the desired intratubular pressure was obtained*; the pressure was measured with a pressure recording cannula inserted in an early proximal tubular segment. Tubular fluid was sampled in the conventional way via a pipette inserted just proximal to the oil block, at a rate which kept the oil column in a fixed position. The experiments were conducted so that one operator adjusted the sampling rate to keep the oil column fixed, while another operator controlled the tubular pressure by adjusting the speed of the continuous oil injection. The sampling times were comparatively long (4–6 min) to eliminate the effect of transient changes in filtration when inducing the counter pressure.

Results

Series one. The results from the pressure measurements are depicted in Table I. At a mean arterial pressure of 118 mm Hg the glomerular capillary pressure averaged 62.6 ± 3.2 mm Hg (mean ± S.D.). The pressures in the early proximal tubules blocked with oil were 37.7 ± 3.9 mm Hg, which by adding the systemic oncotic pressure of 20.3 mm Hg (6% protein) would mean a glomerular pressure of 58.0 mm Hg. A more precise calculation also taking into account the actual albumin-globulin ratio would mean an oncotic pressure of 22 mm Hg (Källskog and Wolgast 1973). The results obtained by the stop-flow method thus corroborate

and coworkers (1969, 1971 b) were also able to withdraw blood samples from the ... end of the efferent arterioles, which are seen on the surface of the kidney branching into the peritubular network with a stellate appearance (welling points or star vessels). By comparing the protein concentration in these samples with that in systemic plasma the filtration fraction could be calculated and thereby also the oncotic pressure in the distal end of the glomerular capillaries. Determinations of the single nephron filtration rate (SNGFR) and the ... glomerular plasma flow also allowed for an evaluation of the dynamics of the glomerular ultrafiltration. The results suggested that the mean net driving force is comparatively low and highly dependent on the plasma flow, due to the fact that a filtration equilibrium ... achieved at the distal end of the glomerular capillaries.

The occurrence of superficially located glomeruli even in the Sprague-Dawley ... though very rare, allowed the same measurements to be made in the present study as ... of Brenner and coworkers. Samples of welling point blood also permitted determination of the filtration fraction. The results show a much higher glomerular capillary pressure, ... about 63 mm Hg, and a single nephron plasma flow of more than twice that obtained in ... mutant Wistar rat.

Methods

All experiments were performed on male Sprague-Dawley rats weighing between 230 and 320 g. The ... were fed on a standard diet (Anticimex, Sweden) and had food and water ad libitum. They ... tized with Inactin® (Chemical Fabr. Promonta, Hamburg, West Germany) intraperitoneally ... 120 mg/100 g b.wt. and placed on a servo-controlled heating pad. The rats were tracheotomized ... left femoral artery and femoral vein were catheterized. The left kidney was exposed via a subcostal incision and placed in a Lucite cup, whereafter the ureter was cannulated. In most cases the dorsal ... the kidney was used for micropuncture, but in some few cases the ventral side was used. During the ... puncture the kidney was superfused with warmed mineral oil.

The experiments were divided into four series. In the *first series* of 9 rats the hydrostatic pressures ... the tubules and the cortical vascular tree were measured, including direct measurements on glomerular capillaries. Such punctures are only possible when a glomerulus is located on the surface of the ... which is rare in the Sprague Dawley rat. This series of experiments therefore comprised animals ... such superficial glomeruli were found incidentally. Superficial glomeruli, when present, are most ... on the ventral side of the kidney. For the purpose of pressure measurement, a servo-controlled ... pressure measuring device as designed by Wiederhielm (1964) and modified by ... taghetti *et al.* (19... used. The punctures were performed with sharpened glass capillaries with ... outer diameter of ... The glomerular capillary pressure was measured by means of direct punctures of the superficial glo... and was verified by injection of Lissamine green into the glomerular capillary tuft. All measurements in which a leak ... into the interstitium, the tubules or Bowman's capsule was established ... The pressures in Bowman's space were recorded by punctures deep into the glomerulus and ... by injections of dye, which under appropriate conditions filled Bowman's space and emptied ... proximal tubule. The hydrostatic pressure in the distal end of the efferent arteriole ... obtained ... pressure ... the welling points, which were easily identified on the surface of the kidney.

The glomerular capillary pressure was also measured indirectly by the stop-flow method as described ... Gertz (1966). For this purpose an early proximal tubule was identified and punctured ... cannula (about 10 μm) filled with coloured mineral oil. The intratubular pressure was continuously ... tored by means of a second cannula inserted proximal to the first one. The oil block ... applied ... filtration had ceased and the pressure was stabilized. The glomerular capillary pressure was calculated by adding this "stop-flow" pressure to the colloid osmotic pressure of the systemic plasma, a protein c... tration of 6% would then correspond to a pressure of 20–21 mm Hg as calculated by the formula ... by Landis and Pappenheimer (1963). This series was completed with some control experiments ... the validity of stop-flow measurements. The oil blocks were applied ... dist..., late proximal, mid-... and early proximal tubules and the stop-flow pressure was recorded just above the oil columns.

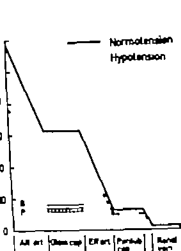


Fig. 1

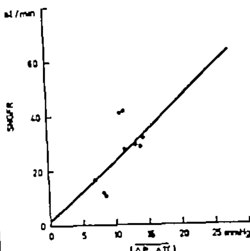


Fig. 2

... Hydrostatic pressures in the superficial renal vasculature, Bowman space and the proximal tubules. ...pper line refers to normotensive conditions and the lower to hypotension of about 80 mm Hg. In the ... curve the glomerular capillary pressures were investigated indirectly by the "stop-flow" method only

...2. Relation between the change of the mean driving pressure, $\overline{\Delta P-\Delta \pi}$, and the SNGFR. For each pair ...intercept on the ordinate and the slope were calculated. The line drawn represents the mean of the individual lines.

...0.28. The superficial SNGFR was 14.1 ± 1.9 nl/min 100 g, and the single nephron effec... plasma flow (SNEPF) 56.4 nl/min 100 g, which will mean a filtration fraction of 0.26. When the perfusion pressure in the renal artery was reduced from 124 mm Hg to 77 mm ..., the total kidney PAH clearance was only reduced to 90% of the control value and the ...e reduction took place in total GFR, *i.e.* these variables will be autoregulated. In con...st, the superficial SNGFR showed a more pronounced reduction, down to 10.4 nl/min ...0 g, *i.e.* 73.8%. Essentially the same reduction was noted for SNEPF. The SNEPF was ...duced to 79.6%.

The filtration fraction estimated from the relation between protein concentration in ...mples withdrawn from welling points and the concentration in systemic plasma, as under...taken in 9 rats in the third series, was 0.27 ± 0.06 (mean ± S.D.). Essentially no change ...curred (0.28 ± 0.04) when the perfusion pressure was reduced from a mean value of 117 mm ...g to 81 mm Hg. Further reductions in perfusion pressure were followed by decreases in the ...tration fraction.

In the *fourth series* it was found that the SNGFR was linearly related to change of the ...ean driving pressure, $\overline{\Delta P-\Delta \pi}$. This result was obtained by determination of the SNGFR ... two pressure levels. The first sampling was undertaken at about a normal pressure and ...e second one during elevated pressure. For the method of calculation of the mean driving ...ressure the reader is referred to Källskog *et al.* (1975 b); the glomerular plasma flow was ...sumed to be the same under both conditions. Fig. 2 shows the results from 18 pairs of

TABLE I. Hydrostatic pressures within the superficial tubular and vascular network in the rat k[illegible] n refers to number of animals. Mean ± S.D.

Arterial pressure mm Hg	Glomerular capillaries mm Hg	Proximal stop flow pressure mm Hg	Welling point pressure mm Hg	Peritub. cap. pressure mm Hg	Prox. tub. pressure mm Hg	Bowman's sp[illegible] pressure mm Hg
118 ± 7.3 n = 9	62.6 ± 3.2 n = 9	37.7 ± 3.9 n = 9	13.9 ± 1.2 n = 8	10.8 ± 1.2 n = 8	13.5 ± 1.3 n = 9	16.0 ± 2.1 n 8

those arrived at directly The stop-flow pressure in the second series (see Table II) amount[s] to 40 mm Hg, thus essentially the same as in the first series. A systematic error might ha[ve] been inherent in the stop-flow measurements. A small degree of filtration could still occ[ur] especially when the blockade was made in late proximal tubules. The pressures obtained i[n] distal tubules and late proximal ones are considerably lower than those in the earlier s[eg]ment The pressure in welling points in the two series amounted to 14 mm Hg. Within the peritubular capillaries the pressure was 11–12 mm Hg.

In the tubular system the pressures within Bowman s space are slightly higher than th[ose] in the proximal tubule, indicating a slight pressure drop which might be located to the ne[ck] of Bowman s capsule. The proximal tubular pressure was estimated at 13.5 mm Hg, wi[th] very small scattering. The data on hydrostatic pressures within the superficial vasculature and tubules are summarized in Fig. 1 The data under normotensive conditions were deriv[ed] from the first series the data from the second series would, however be in complete ac[-] cordance. For hypotensive conditions all data are derived from the second series, and i[t] should be emphasized that the glomerular capillary pressure was only measured indirectly.

For the second series single nephron and total clearance of tritiated inulin and 125I labelled PAH were obtained, in addition to the pressure data. The total GFR was 0.37 ± 0.[06] ml/min as calculated per 100 g b wt The PAH clearance, which may be regarded as a measure of effective renal plasma flow amounted to 1.33 ± 0.19 ml/min 100 g, the filtration fraction

TABLE II Hydrostatic pressures within the superficial tubules and the vascular net ork and data on s[ingle] nephron glomerular filtration (SNGFR), single nephron PAH clearance (SNEPF), total filtration (GFR) and total PAH clearance (EPF), TF/p Inulin in the distal t bules and the single nephron and total fraction (SN FF and FF respectively) n 7 rat kidneys. Each figure is derived from determinations on each rat. Mean ± S.D

Arterial pressure mm Hg	Prox. tub. stop-flow mm Hg	Prox. t b. free flow mm Hg	Welling point mm Hg	Peritubular capillaries mm Hg	SNGFR nl/min 100 g	SNEPF nl/min 100 g
124 ± 4 77 ± 4	40.1 ± 2.7 30.4 ± 2.4	13.9 ± 0.9 1–1 ± 0.9	14.0 ± 1.7 11.2 ± 1.4	1 4 ± 1.8 —	14.1 ± 1.9 10.4 ± –4	56.4 ± 4.0 44.3 – 13.1
	SN FF		GFR ml/min 100 g	EPF ml/min 100 g	FF	TF/p Inulin
124 ± 4 77 ± 4	0.26 ± 0.02 0.24 ± 0.06		0.37 ± 0.06 0.33 ± 0.11	1.33 ± 0.19 1.20 ± 0.29	0.–8 ± 0.01 0.27 ± 0.05	3.6 – 0.34 4.3 – 1.27

...the plasma flow measured with PAH is not representative for the outermost glomeruli. ... tubules belonging to these glomeruli pass far down into the renal parenchyma and will extract PAH from other regions than the outer cortex. It is thus concluded that the ...d method cannot be used for the measurement of plasma flow in superficial glomeruli. ...very interesting finding is that the filtration fraction obtained by 1) the above mentioned ...le nephron effective plasma flow method, 2) the total renal plasma flow and total GFR ...minations, and 3) the welling point method, will show the same value of about 0.27 ...strengthens the assumption of an equal filtration fraction in all the glomerular popula...s. Since the filtration fraction is almost the same during both normo- and hypotension ...ems to be relevant to speak of an autoregulation of the filtration fractions. This is in good ...ordance with the results obtained by Brenner *et al.*

...renner *et al.* concluded that the glomerular filtration rate is directly correlated to the ...merular plasma flow which is a consequence of a filtration equilibrium. As the present ...dy appears to indicate a non-equilibrium, a pressure dependency rather than a plasma ... dependency might be expected. This problem has been evaluated further by Källskog ...al. (1975 b). The effect of the driving pressure on the glomerular filtration rate is further ...ported by the results of the experiments in which a counter pressure was applied in ...man's space, and ... linear relation between the mean driving force and the GFR was ...und. In hypotensive rats, however the plasma perfusion rate was the dominant factor ...ecting GFR. Thus the kinetics of the glomerular filtration process in normotensive mutant ...star rats are comparable with those of hypotensive Sprague Dawley rats.

...In earlier investigations (Källskog *et al.* 1975 a) it has been shown that the outer glomeruli ...not autoregulate with respect to the blood perfusion rate. From SNGFR and the single ...phron filtration fraction under normotensive and hypotensive conditions it was possible ...calculate the blood flow of single glomeruli also in these series of experiments. The results ...dicate the same non-existence of an autoregulation of the blood flow in the superficial ...omeruli.

References

...LLISON, M. E. M., E. M. LIPHAM and C. W. GOTTSCHALK, Hydrostatic pressure in the rat kidney. *Amer. J. Physiol.* 1972. 223. 975–983.

...NDUCCI, V. J. HERRERA-ACOSTA, F. C. RECTOR, D. SELDIN, Effective glomerular filtration pressure and single nephron filtration rate during hydropenia, elevated ureteral pressure, and acute volume expansion with isotonic saline. *J. clin. Invest.* 1971. 50. 2230–2234.

...LANDIZ, R. A. H. ISRAELIT, F. C. RECTOR, D. W. SELDIN, Relation of distal tubular NaCl delivery and glomerular hydrostatic pressure. *Kidney Intern.* 1972. 2. 22–32.

...RENNER, B. K. H. FALCHUK, R. KEIMOWITZ and R. BERLINER, The relationship between peritubular capillary protein concentration and fluid reabsorption by the renal proximal tubule. *J. clin. Invest.* 1969. 48. 1519–1531.

...RENNER, B., J. L. TROY. Postglomerular vascular protein concentration. Evidence for causal role in governing fluid reabsorption and glomerulotubular balance by the renal proximal tubule. *J. clin. Invest.* 1971 a. 50. 336–349.

...RENNER, B. M. J. L. TROY and T. M. DAUGHARTY. The dynamics of glomerular ultrafiltration in the rat. *J. clin. Invest.* 1971 b. 50. 1776–1780.

...RENNER, B. M., J. L. TROY and T. M. DAUGHARTY. Pressures in cortical structures of the rat kidney. *Amer. J. Physiol.* 1972. 222. 246–251.

GERTZ, K. H., J. A. MANGOS, G. BRAUN and H. D. PAGEL, Pressure in the glomerular capillaries of the rat kidney and its relation to arterial blood pressure. *Pflügers Arch. ges. Physiol.* 1966. 288. 369–374.

determinations. For each pair the intercept on the ordinate and the slope were calculat[illegible] The line drawn represents the mean of the individual lines.

The blockade means that the delivery to the distal tubules and thereby the macula de[illegible] region is zero. This would not seem to be of great significance, however since the SNGF[illegible] as determined by proximal sampling corresponds to that obtained on sampling in the dis[illegible] tubules as shown by Persson *et al* (1974) in experiments on rats fed on the same kind of [illegible] The glomerular blood flow was assumed to be unchanged during the blockade.

Discussion

Brenner and coworkers used rats of a mutant Wistar strain, because of the frequent occ[illegible] rence of superficial glomeruli in these rats. However the hydrostatic pressures within t[illegible] glomerulus seem to differ from those found in the more commonly used non-mutant Wi[illegible] and Sprague-Dawley rats. In the Sprague-Dawley strain, which we have used, superfic[illegible] glomeruli suited for micropuncture are found occasionally It seems more reliable to [illegible] data from these kidneys, since the results can be compared with the data obtained in [illegible] micropuncture experiments.

The glomerular hydrostatic pressures obtained in the present investigation are [illegible] higher than those measured by Brenner *et al* Most striking is the fact that the glomer[illegible] capillary pressure of the Sprague Dawley rats exceeds that of the mutant Wistar rats [illegible] more than 15 mm Hg. As the hydrostatic pressure in Bowman s space and the oncotic [illegible] do not differ between the two rat strains, the net driving force seems consequently [illegible] higher in the Sprague-Dawley rats. (See also Allison *et al* 1972.)

In this context the problem of the existence of an equilibrium or a disequilibrium regi[illegible] the filtration process must also be kept in mind The mean net driving pressure under [illegible] motensive conditions can be calculated to be about 20 mm Hg, and during hypote[illegible] about 12 mm Hg. From the direct pressure measurements and the determination of [illegible] filtration fraction a driving pressure of about 13 mm Hg can also be calculated at the [illegible] of the glomerular capillary which means that an equilibrium does not exist under no[illegible] conditions. During hypotension the end-capillary net driving pressure decreased to [illegible] Hg. The implication of the presented results differs from that of the Brenner group an[illegible] be discussed in detail in a forthcoming paper (Källskog *et al.* 1975 b).

The direct pressure measurements in Bowman s space and in the early proximal t[illegible] showed a pressure difference of about 2.5 mm Hg and indicate a flow resistance at the [illegible] tion between Bowman s space and the proximal tubule. This has to be taken into consi[illegible] tion when calculating the driving forces over the glomerular membrane. In this con[illegible] must also be pointed out that the directly measured intracapillary pressure is som[illegible] higher than that obtained by the stop-flow method. The more distally the stop flow pr[illegible] is measured, the greater the difference.

The single nephron effective plasma flow measurements with PAH during normot[illegible] and hypotension indicated an autoregulation of the renal plasma flow which is not [illegible] cordance with the earlier findings of non-autoregulation in the outer parts of the renal [illegible] in the rat (Källskog *et al* 1975 a). This difference is due to the fact that the single ne[illegible]

Acta physiol. scand. 1975. 95. 301–310

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Effects of Hypoxia of 10–45 Seconds Duration on Energy Metabolism in the Cerebral Cortex of Unanesthetized and Anesthetized Rats

By

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Abstract

NORBERG, K., B. QUISTORFF and B. K. SIESJÖ. *Effects of hypoxia of 10–45 seconds duration on energy metabolism in the cerebral cortex of unanesthetized and anesthetized rats.* Acta physiol. scand. 1975. 95. 301–310.

Glycolytic and citric acid cycle intermediates, as well as organic phosphates, were measured in the cerebral cortex of unanesthetized rats following arterial hypoxia (administration of 4–8 % O_2) of 10 and 20 s duration. There were decreases in glucose-6-phosphate and fructose-6-phosphate, and increases in fructose-1,6-diphosphate, dihydroxyacetone phosphate and 3-phosphoglycerate, even before pyruvate accumulated. Since measurements of the lactate concentration showed that there was an increased glycolytic rate, the results demonstrate that phosphofructokinase was activated. The glycolytic changes were accompanied by, and probably due to, minor changes in phosphocreatine, ATP, ADP and AMP. Experiments on anesthetized animals showed that hypoxia for 45 s was accompanied by signs of phosphofructokinase activation, even if tissue P_{CO_2} was kept constant. It is concluded that, irrespective of the tissue CO_2 tension, hypoxia is accompanied by activation of phosphofructokinase which, at least initially, is responsible for increased glycolytic rate.

Recent results (Duffy *et al.* 1972, Bachelard *et al.* 1974, Norberg and Siesjö 1975a and b) have provided new information on the cerebral metabolic changes that occur when animals are exposed to atmospheres containing low concentrations of oxygen ("hypoxic hypoxia"). The following tentative conclusions were drawn. Soon after the induction of hypoxia, there is acceleration of glycolytic rate with accumulation of lactate and pyruvate, and increase in the lactate/pyruvate ratio. As a part of a generalized oxidation/reduction change there is also an increase in the malate/oxaloacetate ratio with a fall in oxaloacetate (OAA) concentration. The latter induces a shift in the aspartate aminotransferase equilibrium, resulting in an initial reduction in α-ketoglutarate (α-KG) level and a sustained reduction in aspartate. When hypoxia is prolonged, the elevated pyruvate concentration induces a shift in the alanine aminotransferase reaction towards alanine formation, as well as an increased rate of

GERTZ, K., M BRANDIS, G BRAUN-SCHUBERT and J W BOYLAN, The effect of saline infusion [illegible] rhage on glomerular filtration pressure and single nephron filtration rate. *Pflügers Arch. ges. Physiol.* 1969 *310* 193–205.

INTAGLIETTA, M., R. F PAWULA and W R. TOMPKINS, Pressure measurements in the [illegible] vasculature. *Microvasc. Res* 1970, *2*, 212–220.

KÄLLSKOG, Ö and M WOLGAST Driving forces over the peritubular capillary membrane in the [illegible] during antidiuresis and saline expansion. *Acta physiol. scand.* 1973, *89*, 116–125.

KÄLLSKOG, Ö L. O. LINDBOM, H. R. ULFENDAHL and M WOLGAST The pressure-flow [illegible] different nephron populations. *Acta physiol scand.* 1975 a. *94* 289–300.

KÄLLSKOG, Ö L. O. LINDBOM, H. R ULFENDAHL and M WOLGAST Kinetics of the glomerular [illegible]tion in the rat kidney A theoretical study *Acta physiol scand.* 1975 b. *95* 191–200.

KOCH, K. M., T DUME, H. H KRAUSE and B. OCHWADT Intratubulärer Druck, glomerulärer Capillardruck und Glomerulumfiltrat während Mannit Diurese. *Pflügers Arch. ges. Physiol.* [illegible]

KRAUSE, H., T DUME, K. M KOCH and B. OCHWADT Intratubulärer Druck, glomerulärer Capillar[illegible] und Glomerulumfiltrat nach Furosemid und Hydrochlorothiazid. *Pflügers Arch. ges. Physiol.* [illegible] 80–89.

LANDIS, E. M and J R. PAPPENHEIMER, Exchange of substances through the capillary wall. *Handbook physiology Circulation II* Waverly Press, U.S.A. 1963 961 1034.

PERSSON, E. and F WRIGHT Reduction of glomerular filtration rate by intrarenal feedback during [illegible]zolamide diuresis. *Fed Proc.* 1974. *33* 347

ROBERTSON, C. R., W M DEEN, J L. TROY and B. M. BRENNER, Dynamics of glomerular ultrafiltration in the rat. III Hemodynamics and autoregulation. *Amer J Physiol.* 1972, *223*, 1191–1200.

WIEDERHIELM, C. A., J W WOODBURY S. KIRK and R. F RUSHMER, Pulsatile pressures in the [illegible]culation of frog's mesentary *Amer J Physiol.* 1964 *297* 173–176.

Unanesthetized animals

nanesthetized animals, weighing 200–220 g, preliminary operation was performed under 2% ane anesthesia, and tail artery catheter was inserted. After 1–2 hrs of recovery the rats ere al- to creep into the cylinder of the freeze-clamping apparatus (see Quistorff 1975), and were kept there restrained position for 10–20 min. During this period of adaptation the cylinder was flushed with room d measurements were made of blood pressure, rectal temperature and of arterial P_{O_2}, P_{CO_2} and pH. ia was then induced for either 10 or 20 by rapid switching to hypoxic gas mixture containing 6% O_2. In preliminary experiments, serial samples were drawn from the artery for determining the time of the changes in arterial gas tensions during the induction of hypoxia.

controls were freeze-clamped at the end of the adaptation period, and the hypoxic animals at the f the 10 and 20 periods of hypoxia. At the time of freeze-clamping, an arterial blood sample was . The performance of the apparatus is such that the part of the rat positioned in the "biopsy region cylinder is cut free and freeze-clamped between precooled aluminium blocks in about 0.1 s. (For of sampling procedure, see Quistorff 1975.) The freeze-clamped slice, which contained the mid-part brain, was immersed in liquid nitrogen and later stored at −80°C. Using burr saw at 30°C, m thick section as then cut off from the surface of the brain section that had been brought in con- th one of the freeze-clamping blocks. Cerebral cortical tissue from this thin section as used for ses.

Anesthetized animals

anesthetized series of animals (hypoxic and control animals, the latter maintained at an arterial P_{O_2} 100 mm Hg), the techniques used were similar to those described previously (Norberg and Siesjö) and the reader referred to that publication for details. The main features were as follows. An- was induced with 2–3 halothane and the animals were tracheotomized, immobilized with tubo- rine chloride and connected to respirator. At the time of tracheotomy the halothane anesthesia was ntinued and the animals were maintained on 70% N_2O and 30% O_2 until hypoxia as induced. An rial catheter was inserted to allow sampling of arterial blood and measurement of blood pressure. The / temperature, as measured in the rectum, was kept close to 37°C. The skin over the head as incised commodate plastic funnel for subsequent freezing of the tissue in situ. At the induction of hypoxia, ygen concentration of the inspired air was decreased so as to give (final) arterial P_{O_2} of about mm Hg, keeping the N_2O concentration constant. The gas mixture administered contained sufficient n dioxide to increase arterial P_{CO_2} by 2–4 mm Hg during the hypoxia. After 45 of hypoxia, the was frozen in situ by pouring liquid nitrogen into the funnel. The frozen tissue was kept at −80°C. tical tissue was then cut out, weighed and extracted at 22°C.

Analytical techniques

and P_{CO_2} in arterial blood were measured at 37°C with microelectrodes. Cortical tissue was dissected, hed and extracted ith HCl-methanol at 22°C. The following metabolites are analysed (in some ts only selected number was studied): phosphocreatine (PCr), creatine, ATP ADP AMP glycogen, ose, glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), fructose-1,6-diphosphate (FDP), droxyacetone phosphate (DHAP), 3-phosphoglycerate (3-PG), pyruvate, lactate, citrate, α-keto- rate (α-KG), succinate, malate, glutamate, aspartate and NH The basic methods were those de- bed by Lowry and Passonneau (1972) and analytical conditions have been given in previous communica- ons (for references, see Folbergrová et al. 1974). The oxaloacetate concentrations were calculated from aspartate aminotransferase reaction, using an equilibrium constant of 6.7 (Krebs 1957).

tistical differences are calculated using Aspin–Welch test or the nonparametric Mann–Whitney test, hen the values ithin the group did not seem to follow normal distribution. The following bols are used p 0.05, ** = p 0.01 and *** = $p < 0.001$

Results

Unanesthetized animals

ese animals were provided with tail artery catheters, and cortical tissue was obtained for alysis by means of "freeze clamping following exposure to about 8 and 6% O_2, respec- ely. Originally the gas with the lower concentration (6%) was administered to one group

CO_2 fixation. As a result, there is an increased alanine concentration and an increase in pool size of tricarboxylic acid cycle (TCA) intermediates.

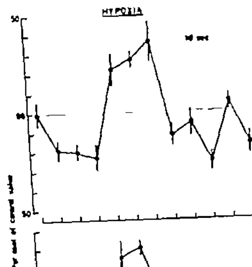
There have been some difficulties in defining the regulatory enzymatic reaction that is responsible for the accelerated glycolysis. Two previous studies were partly devoted to the problem (Duffy *et al* 1972, Bachelard *et al* 1974) but in none could evidence be obtained for an activation of phosphofructokinase (PFK) the enzyme which is considered to exert the main regulatory control of glycolytic rate (Lowry and Passonneau 1964 1966. The results of both groups of workers were consistent with the idea that, in hypoxia, pyruvate kinase may be regulatory However by shortening the period of hypoxia to 45 s, or 1 ... and 45 s, and by allowing the P_{CO_2} to fall spontaneously we could show that an activation of PFK activity is involved in triggering an increased glycolytic rate (Norberg and Siesjö 1975 a) Our tentative explanation for the difference in results between that study and the previous one (Bachelard *et al.* 1974) was as follows. Soon after the induction of hypoxia there is an imbalance between production and utilization of ATP The consequences of this imbalance, *i.e* a moderate increase in ADP (and P_i), and a decrease in phos... lead to PFK activation and increased glycolysis. However the activity of PFK is ... decreased, relative to the activities of hexokinase and of pyruvate kinase, and this "inactivation" of PFK is accelerated by the development of tissue acidosis.

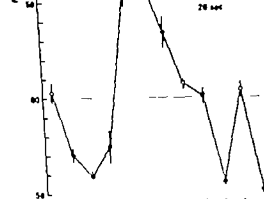
Before a completely satisfactory explanation can be given of the glycolytic events in hypoxia some further information is required *Firstly* previous results were obtained in nitrous oxide anesthesia and since anaesthesia may blunt the tissue response to hypoxia, it would be desirable to study unanesthetized animals. *Secondly* no information is at hand about metabolic changes occurring during the first 45 s period of hypoxia. Thus, tissue analyses performed at the very induction of tissue hypoxia might reveal larger changes in labile phosphates than are observed after 45 s in anesthetized animals. Such information is also essential for establishing that the increased pyruvate and lactate concentrations are consequences of increased glycolysis, and not just due to reduced pyruvate oxidation. Thus, if it could be shown that PFK is activated before pyruvate and lactate have accumulated, this problem would be solved. *Thirdly* when the arterial P_{CO_2} is allowed to fall during the hypoxia there is in all probability an alkaline shift of intracellular pH during the first minutes (Norberg and Siesjö 1975 a). Since alkalosis is believed to stimulate glycolysis (Delcher and Shipp 196... becomes difficult to decide whether or not it contributes to PFK activation. Accordingly it would be desirable to establish that PFK activation occurs at constant CO_2 tension in the tissue.

The present experiments were designed to provide information on the above points using a "freeze clamping" technique (Quistorff 1975) it was possible to obtain cerebral cortical tissue for analysis from unanesthetized animals, and to study hypoxia of only ... 20 sec duration. Activation of glycolysis at an unchanged tissue CO_2 tension was studied in anesthetized animals following a hypoxic period of 45 sec.

Methods

The experiments were performed on male Wistar rats that had free access to water and rat pellets ... studied. There were two main series of animals—unanesthetized and anesthetized—and these ... described separately





2 Influence of hypoxia (administration of 6-8 % O_2) of 10 (20) s duration upon cerebral concentration of glycolytic and citric acid cycle metabolites. Values are means ± S.E.M. as per cent of the control values that are given at the bottom. Filled symbols denote values significantly different from the controls ($p < 0.05$) ... were used. Gly = glycogen, Glc = glucose, G-6-P = glucose-6-phosphate, F-6-P = fructose-6-phosphate, FDP = fructose-1,6-diphosphate, DHAP = dihydroxyacetone phosphate, 3-PG = 3-phosphoglycerate, Pyr = pyruvate, Cit = citrate, α-KG = α-ketoglutarate, Mal = malate, OAA = oxaloacetate.

...n. The latter was measured in 4 control animals and in 4 animals exposed to 6% O for ...s. The blood glucose concentrations in these groups were 10.2 ± 0.3 and 9.5 ± 0.4 μmol/g, respectively and the corresponding tissue to blood glucose concentration ratios were 0.33 ± ...4 and 0.32 ± 0.03, respectively. Thus, there were small, if any, changes in the ratios.

In the hypoxic animals, there were decreases in G-6-P, F-6-P, α-KG and (calculated) OAA, and increases in FDP, DHAP and 3-PG. These results are entirely consistent with those obtained previously (Norberg and Siesjö 1975 a and b) and demonstrate that, in unanaesthetized animals, activation of PFK occurs already 10 s following induction of hypoxia (see Discussion).

Although there were signs of an activation of PFK at 10 s of hypoxia, there was neither an increase in tissue lactate content at that time, nor significant elevations of lactate/pyruvate

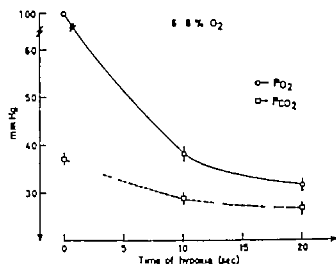


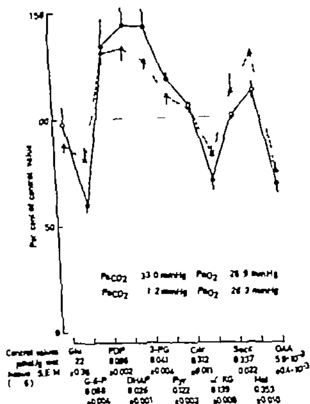
Fig. 1 Time course of changes in P_{O_2} and P_{CO_2} during administration of 6–8% O_2 to unanesthetized rats. values are means ± S.E.M.

of animals (n = 4) studied after 10 s of hypoxia, in an attempt to maintain Pa_{O_2} co at 10 and 20 s of hypoxia. However the Pa_{O_2} obtained was not significantly different that observed with 8% O_2. For this reason, all values obtained at 10 s were pooled. also the two control groups were pooled, there were 9 control animals, 10 hypoxic studied at 10 s, and another 6 studied at 20 s. The mean P_{O_2} and P_{CO_2} values obtain shown in Fig. 1 At 10 and 20 s, the mean arterial P_{O_2} was 38.0 and 31.5 mm Hg, and P_{CO_2} was reduced by 8–10 mm Hg.

Fig. 2 shows the pattern of changes in glycolytic and citric acid cycle intermediate and 20 s of hypoxia as compared to the control values, the absolute values of wh given at the bottom. It should be noted that the control values obtained in unanest animals after freeze-clamping were very close to those obtained with nitrous oxide anc and surface freezing through the bone (see Norberg and Siesjö 1975 a, and b, and Although the changes observed at 20 s were somewhat more marked than those seen the pattern was consistent. There were no changes in glycogen but significant reduct glucose content. However this reduction should be interpreted with caution since, given animal, the glucose content in the tissue must depend on the blood glucose con

TABLE I. Cerebral cortex concentrations of ATP ADP AMP PCr Cr and NH in unanesthetized following administration of gases with low oxygen content (6–8% O_2) for 10 or 20 s. Th expressed in μmol/g of wet tissue, are means ± S.E.M.

Time of hypoxia	n	ATP	ADP	AMP	PCr	Cr	NH
0	9	2.75 ±0.04	0.399 ±0.012	0.060 ±0.005	3.17 ±0.11	6.84 ±0.16	0.20. ±0.011
10 s	10	2.67 ±0.05	0.421 ±0.015	0.068 ±0.007	2.79 ±0.07	7.28 ±0.26	0.196 ±0.021
20 s	7	2.61 ±0.03	0.475 ±0.018	0.111 ±0.012	2.15 ±0.09	8.26 ** ±0.22	0.27 ±0.027



4. Influence of hypoxia (re- ... arterial P_{O_2} to 26–27 ... Hg) upon cerebral cortex ... of glycolytic and ... acid cycle intermediates in ... anesthetized with nitrous (70 %). The values obtained ... normocapnic animals (P_{aCO_2} ... mm Hg) were compared to ... previously obtained in ... capnic animals (Norberg and ... 1975 ... and b). The values ... means ± S.E.M. in per cent of ... trols, that are given at the ... Filled symbols denote ... significantly different from ... controls (p ... 0 05). Succ ... For further abbreviations, ... Fig. 2.

... poxic animals the arterial P_{CO_2} could be kept at 41.2 ±0.7 mm Hg, values that were slightly ... her than those of the controls (37.9 ±0.4 mm Hg). The difference ensured that tissue P_{CO_2} ... as kept at, or just above, the normal value even if there was an increase in cerebral blood ... ow (see Bachelard *et al.* 1964).

Fig. 4 shows the changes observed in glycolytic and citric acid cycle metabolites in nor

... II. Influence of reduction in arterial P_{O_2} to 26 mm Hg on tissue metabolites in normocapnic rats, anesthetized with nitrous oxide. The duration of hypoxia was 45 s. Values are means ± S.E.M. (n 6 in both groups)

	Control	Hypoxia Normocapnia
[illegible]	4 35 ± 0 06	3.79 ± 0 04***
[illegible]	6 04 ± 0 05	6.56 ± 0.10**
TP	3.02 ± 0.03	2.96 ± 0 05
DP	0 299 ± 0 006	0.304 ± 0 005
MP	0 026 ± 0 001	0 030 ± 0 001
actate	1.32 ± 0.05	1.88 ± 0 07***
act/Py	10 8 ± 0.3	14 1 ± 0.3***
lutamate	13.4 ± 0 2	13 8 ± 0.1
spartate	3.85 ± 0 11	3.59 ± 0.13
Mal/OAA	60 ± 3	103 ± 4***

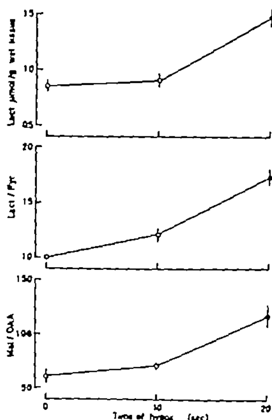


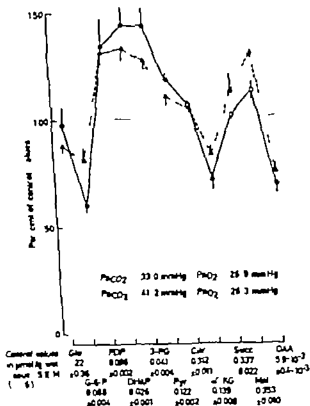
Fig. 3 Influence of hypoxia (administration of 8% O_2) on cerebral cortex concentrations of la... and on calculated lactate/pyruvate and ma... oxaloacetate ratios in unanesthetized animals. M... ±S.E.M. Filled symbols denote values signific... different from the controls ($p < 0.05$).

and malate/oxaloacetate ratios (Fig. 3) At 20 s, the lactate content and the ratios for redox couples were significantly increased.

Table I illustrates the tissue concentrations of ATP ADP AMP PCr creatine and NH... It should be noted that, although "freezeclamping" introduces a very short delay betwe... terruption of cerebral circulation and freezing of the tissue, the values for organic phosp... were less "optimal" than those obtained with surface freezing without previous inter... tion of circulation (see Norberg and Siesjö 1975 a and below). Thus, PCr was about ... µmol/g lower ADP about 0.1 µmol/g higher and AMP about twice as high as with surf... freezing. Although these differences indicate that there were some autolytic changes ... freeze-clamping, the values are sufficiently close to the "normal" to allow comparison b... tween control and hypoxic brains. Thus, at 20 s, there was a highly significant decrease ... PCr and increase in Cr there were significant increases in ADP and AMP and a s... reduction in ATP The data obtained at 10 s indicated that smaller but similar changes ... occurred but only the decrease in PCr was statistically significant. The NH^+ concentr... was normal at 10 s of hypoxia but significantly increased at 20 s. The data allow a discus... of activation of glycolysis in terms of changes in concentration of glycolytic modulators ... Discussion).

Anesthetized animals

In the hypoxic animals, the arterial P_{O_2} was reduced to 26.3 ± 0.6 mm Hg as compared ... 144 ± 6 mm Hg in the controls. By addition of CO_2 to the gas mixture administered to ...



4. Influence of hypoxia (reduction in arterial P_{O_2} to 26–27 mm Hg) upon cerebral cortex concentrations of glycolytic and citric acid cycle intermediates in rats anaesthetized with nitrous oxide (70 %). The values obtained in normocapnic animals (P_{aCO_2} ... mm Hg) were compared to values previously obtained in hypercapnic animals (Norberg and Siesjö 1975 a and b). The values are means ± S.E.M. in per cent of controls, that are given at the bottom. Filled symbols denote values significantly different from controls ($p < 0.05$). Succ = succinate. For further abbreviations, see Fig. 2.

...poxic animals the arterial P_{CO_2} could be kept at 41.2 ± 0.7 mm Hg, values that were slightly higher than those of the controls (37.9 ± 0.4 mm Hg). The difference ensured that tissue P_{CO_2} was kept at, or just above, the normal value even if there was an increase in cerebral blood flow (see Bachelard *et al.* 1964).

Fig. 4 shows the changes observed in glycolytic and citric acid cycle metabolites in nor...

TABLE II Influence of reduction in arterial P_{O_2} to 26 mm Hg on tissue metabolites in normocapnic rats, anaesthetized with nitrous oxide. The duration of hypoxia was 45 s. Values are means ± S.E.M. (n = 6 in both groups)

	Control	Hypoxia Normocapnia
...P	4.35 ± 0.06	3.79 ± 0.04
...	6.04 ± 0.05	6.56 ± 0.10**
ATP	3.02 ± 0.03	2.96 ± 0.05
ADP	0.299 ± 0.006	0.304 ± 0.005
AMP	0.026 ± 0.001	0.030 ± 0.001
Lactate	1.32 ± 0.05	1.88 ± 0.07**
Lact/Pyr	10.8 ± 0.3	14.1 ± 0.5**
Glutamate	13.4 ± 0.2	13.8 ± 0.1
Aspartate	3.85 ± 0.11	3.59 ± 0.13
Cal OAA	60 ± 3	103 ± 4***

mocapnic animals (triangles) and compares them to the corresponding changes at an identical P_{O_2} in animals that became hypocapnic during the 45 s period of (circles, values taken from Norberg and Siesjö 1975 a). The values for F-6-P were since they varied unduly in the normocapnic group. The results demonstrate that the of changes observed in the normocapnic animals was very similar to that previously in hypocapnic animals. Thus, activation of glycolysis at the PFK step seems to occur the tissue P_{CO_2} is held constant (see Discussion).

Table II shows the organic phosphates, the lactate contents, the lactate/pyruvate as well as the glutamate and aspartate concentrations. From the latter and from the concentrations (see Fig. 4), it was possible to derive OAA concentrations and, thereby malate/OAA ratios (see Methods). There was a highly significant decrease in PCr, crease in creatine, and a small rise in AMP but no significant change in ADP. The pyruvate and malate/OAA ratios were significantly increased.

Discussion

Freezing techniques suitable for studying cerebral metabolic changes during short periods of hypoxia in unanesthetized animals include the "brain blower" developed by Veech et al. (1973) and the "freeze-clamping" method (Quistorff 1975). Data obtained on control animals in the present series indicate that the techniques are comparable. Thus, although Veech *et al.* (1973) reported somewhat higher values for PCr, the ADP concentration was higher and the AMP values were similar. However, both these techniques give values for organic phosphates that are somewhat less "optimal" than those obtained by the standard freezing technique of this laboratory (Pontén *et al.* 1973). Probably these differences are due to the fact that, in freeze blowing or freeze-clamping, the circulation is interrupted before freezing occurs, and even if the delay before freezing is only 1–2 s, minor autolytic changes seem to occur. As remarked, these changes are so small, though, that they should not in any way invalidate the conclusions drawn from the present study. It should also be noted that freeze-clamping does not seem to affect the concentrations of glycolytic or citric acid cycle intermediates.

The present results, which extend those previously published (Norberg and Siesjö 1975 a and b), provide answers to the questions raised in the introduction. The main results concern the activation of glycolysis, and the "initial" changes occurring in citric acid cycle intermediates and organic phosphates. The glycolytic changes observed can be interpreted according to the postulate of Krebs (1957) which states that when the flux through the pathway is increased, there should be a decrease in the concentration of the substrate of a regulatory enzyme. In the previous studies it was not possible to prove that the flux was increased in hypoxia, but the present results show that this must be the case. Between 10 and 20 s of hypoxia the total sum of glycolytic intermediates plus lactate increased with 0.57 μmol/g, of which lactate formation accounted for 0.55 μmol/g. The oxygen consumption of the cerebral cortex in Wistar rats is about 4.5 μmol/(g min) (Norberg and Siesjö 1974) which corresponds to a glucose consumption of about 0.8 μmol/(g min). Thus, since the normal production of pyruvate should be about 1.6 μmol/(g min), or about 0.25 μmol/g for a 10

cond, accumulation of 0.55 μmol/g of lactate between 10 and 20 s shows that glycolysis was lerated. Therefore, the glycolytic rate must ha e been at least doubled. These arguments d be wrong if pyruvate were formed in large amounts via the alanine aminotransferase tion or from citric acid cycle intermediates in a reversal of CO_2 fixation reactions. ever this possibility can be excluded since hypoxia is accompanied by an increase in alanine concentration, and by an increase in the sum of citric acid cycle intermediates rberg and Siesjö 1975 b). Thus, in view of these arguments, and of the fact that signs of K activation occurred before pyruvate had accumulated, it can be concluded that ac ration of glycolysis was present.

n view of the fact that glycolytic rate was increased, and that there were decreases in P and F-6-P it can be concluded that PFK was activated. In the present unanesthetized mals, this occurred already 10 s after the induction of hypoxia when the pyruvate and ate concentrations had not yet increased. In the previous study (Norberg and Siesjö 5 a) there were ery small changes in organic phosphates, and it was concluded that if ation of PFK is secondary to a perturbation of the energy state, the enzyme must pond to very small changes in PCr ADP and P The present experiments confirm this clusion since only PCr and creatine were significantly altered at 10 s.

The present results showed that changes in the concentrations of KG and OAA develop l paral with those of glycolytic intermediates. At 10 and 20 s, malate was not significantly anged, and the increase in malate/OAA ratio was thus due to a fall in OAA concentration. e present findings give support to the previously proposed sequence of events during poxia (Duffy *et al.* 1972, Norberg and Siesjö 1975 a and b). Thus, it seems probable that increased malate/OAA ratio, by causing a relative reduction in OAA concentrations, is companied by shift of the aspartate aminotransferase reaction towards glutamate forma n, giving rise to reduction in the aspartate and α-KG levels. Secondarily this decrease α-KG can, together with a subsequent increase in pyruvate, also explain that there is ntually a shift in the alanine aminotransferase reaction with a rise in alanine concentration.

As judged from the changes in PCr ATP ADP and AMP (Table I), the perturbation of e energy state of the tissue in the present unanesthetized animals was larger than that served previously on animals anaesthetized with nitrous oxide (Norberg and Siesjö 1975 a). owever tw facts indicate that this is not necessarily the effect of anesthesia. First, the esent results pertain to only 10 and 20 s of hypoxia. There are results which suggest that a compensatory increase in cerebral blood flow (CBF) develops during the first 2 min orgström *et al.* 1975). Thus, there may be a relatively large initial derangement of cerebral ergy state which is later diminished due to hyperemia. Second, in preliminary experiments th the application of the present freezing technique, it was found that semi-steady state poxia (Pa_{O_2} about 25 mm Hg for 30 min) was associated with a fall in PCr, but no detect ble changes in ATP ADP or AMP These results are in accordance with previous ones btained in N_2O anesthesia (Bachelard *et al.* 1974). In view of these facts, we tentatively onclude that anesthesia with N_2O does not significantly influence the response of the tissue o hypoxia.

The experiments carried out on anesthetized animals to evaluate the effect of CO_2 tension demonstrate that, at 45 s, there are signs of activation of PFK even if tissue P_{CO_2} is held

mocapnic animals (triangles) and compares them to the corresponding changes of at an identical P_{O_2} in animals that became hypocapnic during the 45 s period of h (circles, values taken from Norberg and Siesjö 1975 a). The values for F-6-P were c since they varied unduly in the normocapnic group. The results demonstrate that the p of changes observed in the normocapnic animals was very similar to that previously ob in hypocapnic animals. Thus, activation of glycolysis at the PFK step seems to occur the tissue P_{CO_2} is held constant (see Discussion)

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Effects of Histamine, Vasopressin, and Angiotensin II on Hepatosplanchnic Hemodynamics, Liver Function, and Hepatic Metabolism in Cats

By

N. KRARUP

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Abstract

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[illegible] infusions of histamine (5 μg/kg·min), vasopressin (10 mU/kg min), or angiotensin II (5 μg/kg·min) were given to fasting cats. Hepatic arterial flow was decreased 30 % by histamine, increased [illegible] % by vasopressin, and not significantly affected by angiotensin, [illegible] portal venous flow was [illegible]ased 25 % by histamine, decreased 40 % by vasopressin, and not significantly affected by angiotensin. [illegible] hepatic arterial conductance was decreased about 25 % by histamine and angiotensin, and not significantly affected by vasopressin. The gastrointestinal conductance was decreased about 40 % by vasopressin [an]d angiotensin, and increased 25 % by histamine. The conductance in the intrahepatic low pressure vessels [was] not affected by histamine and vasopressin, but decreased 25 % during the infusion of angiotensin. [Th]ese hemodynamic effects, however, are not accompanied by changes in the liver function or hepatic [me]tabolism as judged from the splanchnic elimination of ethanol, the hepatic uptake and excretion of ICG, [the] hepatic oxygen consumption, and lactate and ketone production. This indicates that the functional [ca]pacity of the liver and thereby the number of sinusoids perfused is not markedly influenced by these [dru]gs. Vasopressin caused [a] decrease in the oxygen consumption and an increase in the lactate production [in] the prehepatic splanchnic area, [wh]ich may be due to [a] redistribution of the gastrointestinal blood flow.

[D]espite numerous studies on the hepatosplanchnic circulatory response to vasoactive [su]bstances possible relationships between drug effects on vascular functional and meta[bo]lic parameters in the splanchnic area has only been sparsely investigated (Greenway and [S]tark 1971).

In previous experiments the effects of catecholamines, glucagon and dibutyryl-cyclic [AM]P on the splanchnic vascular system were found to be independent of effects on the [h]epatic metabolism and liver function (Krarup 1973, Krarup and Larsen 1974, Krarup, [L]arsen and Munck 1975).

In the present experiments the effects of continuous i. v. infusions of histamine, vaso[p]ressin and angiotensin II on hepatosplanchnic hemodynamics were tested together with a [si]multaneous investigation of the liver function and splanchnic metabolism.

Liver function was judged from the splanchnic elimination of ethanol, the secretion of

constant. There is thus no principal difference in response between normocapnic and capnic animals. In all probability the pattern typical of PFK activation disappear rapidly in normocapnic animals because the developing tissue acidosis retards the of PFK, relative to that of other regulatory enzymes.

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References

BACHELARD H. S., L. D. LEWIS, U. PONTÉN and B. K. SIESJÖ, Mechanisms activating glycoly brain in arterial hypoxia. *J. Neurochem.* 1974. 22. 395–401

BORGSTRÖM, L., H. JOHANNSSON and B. K. SIESJÖ, The relationship between arterial P_{O_2} and cer flow in hypoxic hypoxia. *Acta physiol. scand.* 1975. 93. 423–432.

DELCHER, H. K. and J. C. SHIPP. Effect of pH, pCO_2 and bicarbonate on metabolism of glucose b rat heart. *Biochim. biophys. Acta (Amst.).* 1966. 121. 250–260.

DUFFY T. E., S. R. NELSON and O. H. LOWRY. Cerebral carbohydrate metabolism during acu and recovery. *J. Neurochem.* 1972. 19. 959–977

FOLBERGROVÁ, J., B. LJUNGGREN, K. NORBERG and B. K. SIESJÖ, Influence of complete ischemia c tic metabolites, citric acid cycle intermediates, and associated amino acids in the rat cereb *Brain Res.* 1974. 80. 265–279

KREBS, H. A., Equilibria in transamination systems. *Biochem. J.* 1953. 54. 8.–86.

KREBS, H. A., Control of metabolic processes. *Endeavour* 1957. 16. 125–132.

LOWRY O. H. and J. V. PASSONNEAU. The relationship between substrates and enzymes of gl brain. *J. biol. Chem.* 1964. 239. 31–42.

LOWRY O. H. and J. V. PASSONNEAU. Kinetic evidence for multiple binding sites on phosphofru *J. biol. Chem.* 1966. 241. 2268–2279

LOWRY O. H. and J. V. PASSONNEAU. *A Flexible System of Enzymatic Analysis.* Academic Press, and London 1972.

NORBERG, K. and B. K. SIESJÖ. Metabolism of oxygen, glucose, lactate and pyruvate in the rat br *J. Neurochem.* 1974. 22. 1127–1129

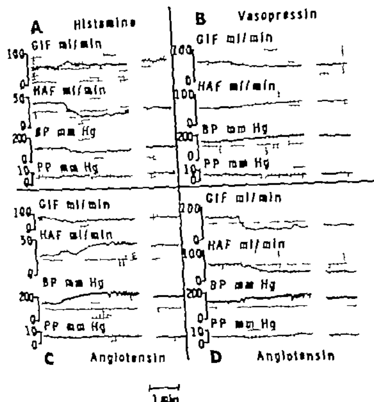
NORBERG, K. and B. K. SIESJÖ, Cerebral metabolism in hypoxic hypoxia. I. Pattern of activatio lysis. *Brain R.s.* 1975 a. 86. 31–44

NORBERG, K. and B. K. SIESJÖ, Cerebral metabolism in hypoxic hypoxia. II. Citric acid cycle int and associated amino acids. *Brain Res.* 1975 b. 86. 45–54.

PONTÉN, U., R. A. RATCHESON, L. G. SALFORD and B. K. SIESJÖ, Optimal freezing conditions f metabolites in rats. *J. Neurochem.* 1973. 21. 1127–1138.

QUISTORFF B., Mechanical device for rapid removal and freezing of liver or brain tissue from v tized and nonparalyzed rats. *Analyt. Biochem.* 1975. In press.

VEECH, R. L., R. L. HARRIS, D. VELOSO and E. H. VEECH, Freeze-blowing: a new technique fo of brain in vivo. *J. Neurochem.* 1973. 20. 183–188.



1. The effects of continuous infusions of histamine, vasopressin, and angiotensin on gastrointestinal (portal venous) flow (GIF), hepatic arterial flow (HAF), mean arterial blood pressure (BP), and portal venous pressure (PP). The drug infusions starts at the beginning of the curves, and the last part of the curves to steady state levels during the drug infusions

ed (Fig. 1 A). This was accompanied by a more pronounced decrease in hepatic arterial blood flow (from 15 ± 1 ml/kg min to 11 ± 1 ml/kg min when steady state in the infusion period was attained). Despite the decrease in arterial blood pressure, portal venous flow increased (from 18 ± 4 to 22 ± 4 ml/kg min). EHBF averaged 31 ± 2 ml/kg min in the control period, and during histamine infusion a value of 32 ± 2 ml/kg min was found. Portal venous pressure varied nearly in parallel with the portal venous flow. The hepatic arterial conductance decreased, gastrointestinal conductance increased and portal venous conductance remained nearly unaltered (Fig. 2).

2. *Vasopressin.* The infusion of vasopressin 10 mU/kg min caused a rise in arterial blood pressure which within a few minutes levelled off about 20% above control pressure (Fig. 1 B). Hepatic arterial flow increased slightly more than arterial blood pressure (from 12 ± 1 to 16 ± 2 ml/kg min). Despite the increase in arterial blood pressure portal venous flow decreased (from 18 ± 1 to 11 ± 1 ml/kg min). In the control period EHBF averaged 33 ± 4, and during vasopressin infusion a value of 29 ± 4 was found. Portal venous pressure varied

bile and the hepatic uptake and excretion of Indocyanine Green (ICG). The sp
metabolism was followed by determinations of the oxygen consumption and uptak
put of lactate, pyruvate, ketone bodies and glucose in the splanchnic area.

Methods

15 fasting cats, anesthetized with chloralose (50 mg/kg) and an initial dose of Nembutal (30 m
used for the experiments. The methods employed were as previous described (Krarup 1973 a). C
for infusion, blood sampling and pressure recordings were placed in the femoral vein and ar
hepatic vein and the portal vein. Electromagnetic flowprobes were placed around the portal vein a
mon hepatic artery after ligation of the right gastroduodenal artery. Bile was collected from
in the choledochus after ligation of the cystic duct.

At the end of the operation priming doses of ethanol (6.5 mmol/kg) and ICG (300 μg/kg) we
into a femoral vein, followed by continuous infusions of ethanol (36 μmol/kg min), ICG (3 μg/k
and taurocholate (0.2 μmol/kg/min). After a recovery and equilibration period of 90 minutes blood s
from a femoral artery and a hepatic vein were drawn at 15 min intervals for determination of the con
tions of ethanol, ICG lactate (L), pyruvate (P), β-hydroxybutyric acid (HB), acetoacetate (Ac), g
and oxygen. Samples from the portal vein for determination of oxygen saturations and—in the
vasopressin experiments—lactate were also drawn. Bile samples were collected for determination o
flow and ICG and electrolyte concentrations. Following a 60 min control period, histamine (5 μg/kg
vasopressin (10 mU/kg min) or angiotensin (0.5 μg/kg min) were given intravenously to groups of
each, and the parameters were followed in another 60 min period.

Calculations

The splanchnic uptake or output of the different substances were calculated from the arterio-he
venous concentration differences and the estimated hepatic blood flow (EHBF) determined by mea
ICG using the Fick principle. The gastrointestinal—including the splenic—oxygen consumption and la
output were calculated from the portal venous flow and the arterio-portal gradients of oxygen and la
The hepatic oxygen consumption was calculated as the difference between the splanchnic and the ga
intestinal oxygen consumption. Clearance and extraction ratio of ICG were calculated by dividing
amount of ICG eliminated and the arterio-hepatic venous ICG difference by the arterial plasma concen
tion of ICG. The vascular conductances in the gastrointestinal area (GIC), the hepatic artery (H
and the intrahepatic low pressure vessels (PVC) were calculated as

$$\mathrm{GIC} = \frac{\mathrm{GIF}}{\mathrm{BP} - \mathrm{PP}} \ \mathrm{ml} \quad \mathrm{kg} \qquad \mathrm{min}^{-1} \times \mathrm{mm\ Hg}^{-1}$$

$$\mathrm{HAC} = \frac{\mathrm{HAF}}{\mathrm{BP}}$$

$$\mathrm{PVC} = \frac{\mathrm{GIF}}{\mathrm{PP}}$$

(GIF gastrointestinal (= portal venous) flow HAF hepatic arterial flow BP mean arterial blood pres
PP portal venous pressure). In accordance with previous findings (Krarup 1973 b) hepatic venous pre
was not statistically different from zero. As, in preliminary series, neither of the drugs were found t
influence hepatic venous pressure, this was set as zero in the calculations.

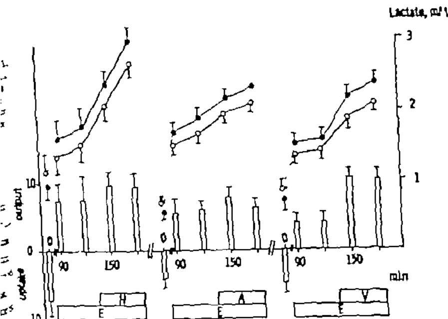
Statistical procedures

The effects of the drugs were tested by Student t test for paired comparisons.

Results

Hepatosplanchnic hemodynamics.

1 *Histamine*. The infusion of histamine 5 μg/kg min caused an immediate decrease i
arterial blood pressure, which in the course of few minutes returned towards the contr



1. The concentrations of lactate in arterial (○) and hepatic venous (●) blood, and the splanchnic in/output of lactate. The bars indicate S.E., E ethanol infusion, H, A, and V the infusions of histamine, ...otensin, and vasopressin.

...teady arterial lactate concentrations were not obtained in the control periods, but as is ...dent from Fig. 3, histamine and vasopressin, in contrast to angiotensin, caused a further ...rease in the lactate concentrations. Histamine and angiotensin did not affect the splanch... lactate output significantly whereas the infusion of vasopressin increased the splanch... lactate output by 100%. In two of the vasopressin experiments portal venous lactate ...centrations were measured. The gastrointestinal lactate output which was insignificant ... the control period increased to about 6 µmol/kg min when vasopressin was given. This ...counted fully for the increase in splanchnic lactate output, thus the hepatic production ... lactate was not affected by vasopressin. Before as well as during the drug infusions the ...patic venous pyruvate concentrations were so small that the L/P ratio in hepatic venous ...lood could not be calculated with sufficient accuracy. There was a splanchnic uptake of ...ruvate of about 1.0 µmol/kg min before as well as during the drug infusions. Infusion ... the vasoactive drugs had no significant effects on the splanchnic production of ketone ...dies and neither was the hepatic venous HB/Ac ratio affected. Histamine infusion was ...lowed by an immediate, pronounced (from 15 to 35 µmol/kg min) but shortlasting (15 ...in) increase in the splanchnic glucose output, whereas neither vasopressin nor angiotensin ...d measurable effects on the glucose concentration and splanchnic glucose output.

Discussion

...he present hemodynamic effects of the drugs agree with previous findings (for review see ...eenway and Stark 1971), except for the constriction in the hepatic arterial bed observed

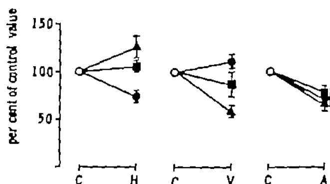


Fig. 2. The vascular conductances in the gastrointestinal area (▲), the hepatic artery (●), and in the hepatic low pressure vessels (■) during the infusion of histamine (H), vasopressin (V) and angiotensin (A), expressed as per cent of the values in the control period. The bars indicate S.E.

nearly in parallel to the portal venous flow The hepatic arterial and portal venous conductances were not significantly affected and the gastrointestinal conductance decreased.

3 *Angiotensin* The infusion of angiotensin 0 5 μg/kg min caused an immediate rise in arterial blood pressure, initially accompanied by cardiac arrhythmia. In the course of 5 min the arrhythmia disappeared, and the arterial blood pressure levelled off about ... above control level. In 4 of the cats hepatic arterial flow increased, somewhat less than arterial blood pressure, and portal venous flow and pressure either remained unaltered or were slightly decreased (Fig. 1 C) In the last cat with a small pressure response to angiotensin, hepatic arterial as well as portal venous flows were markedly reduced, whereas increase in portal pressure was observed (Fig. 1 D). For the whole group hepatic arterial flow portal venous flow and portal pressure in the control period averaged 18 ± 2, 1... (ml/kg min) and 5 8 ± 0.5 (mm Hg) respectively During angiotensin infusion hepatic arterial flow averaged 20 ± 3 ml/kg min, portal flow 16 ± 1 ml/kg min and portal pressure 7 0 ± 0.6 mm Hg. EHBF in the control period averaged 32 ± 4 ml/kg min and was not significantly changed by angiotensin (34 ± 4 ml/kg min). Both the hepatic arterial, gastrointestinal and the portal venous conductance decreased (Fig. 2).

Liver function

None of the drugs influenced the splanchnic elimination rate of ethanol. Histamine and angiotensin had no effect on the plasma clearance and extraction ratio of ICG, whereas vasopressin caused a 10 per cent decrease in ICG clearance ($p < 0.01$) without significant effect on the extraction ratio of the dye. The biliary recovery of ICG was nearly complete (95%) and not changed by the drug infusions. All drugs caused a 10 per cent decrease in bile flow The biliary concentrations of sodium, potassium, chloride, and bicarbonate were not affected.

Splanchnic metabolism

Histamine and angiotensin had no effect on the gastrointestinal and hepatic oxygen consumption. During infusion of vasopressin the gastrointestinal oxygen extraction increased (portal venous oxygen saturation percent was 56 ± 9 in the control period, 46 ± 4 during vasopressin infusions) but not enough to compensate for the decrease in gastrointestinal flow so that the gastro-intestinal oxygen consumption decreased (from 47 ± 5 to 3... μmol/kg min). The hepatic oxygen consumption was not affected by vasopressin.

obic glycolysis as surmised from the decrease in gastrointestinal oxygen consumption, is probably not a generalized hypoxia in the gastrointestinal area, as the oxygen satur of the portal venous blood was only moderately decreased, but may be due to redistri n of the gastrointestinal flow from nutritive vessels to arterio-venous shunts. Hista apparently stimulates hepatic glycogenolysis, whereas angiotensin and vasopressin no such effect, the latter in contrast to findings in perfused rat livers (Hems and Whit 973).

Conclusion

ion of histamine, asopressin and angiotensin at doses producing changes in the tosplanchnic hemodynamics ha e no significant effect on liver function as judged the elimination rate of ethanol and the hepatic uptake and excretion of ICG whereas ght decrease in the production of ductular bile may result. Nor do the drugs affect the tic oxygen consumption or lactate production. This indicates that the number of per d sinusoids in the liver is not changed by the drugs, and that redistribution of the intra- tic blood flow leading to localized hypoxia is not elicited. Vasopressin may cause a stribution of gastro-intestinal flow from nutritive channels to arteriovenous shunts.

skilled technical assistance of Mrs J. Justesen and Mrs L. B. Nielsen is gratefully acknowledged.

References

rs, M. M D S Sclar, J R McNeill and C. V Greenw y Vasopressin and angiotensin on resist ce vessels of spleen, intestine and liver Amer J Physiol. 1970. 218. 1704–06.

ew C V and R. S ark, Hepatic vascular bed Physiol. Rev. 1971. 51 23–65.

s, D. A and P D. Whitton, Stimulation by vasopressin of glycogen breakdow and gluconeogenesis the perfused rat liver Biochem J 1973 136. 705–09.

sen, K R L Ranek and N Tygstrup Liver function and blood flow in normal man during fusion of asopressin Scand J clin Lab. Invest 1969. 24 279–84.

rup N The effect of noradrenaline and adrenaline on hepatosplanchnic hemodynamics, functional pacity of the liver and hepatic metabolism. Acta physiol. scand. 1973 87 307–19

rup N The effect of hemorrhage on hepatosplanchnic hemodynamics, liver function and hepatic metabolism. Acta physiol. scand. 1973 b. 89. 260–77

rup N. and J A. Larsen, The effect of glucagon on hepatosplanchnic hemodynamics, functional pacity and metabolism of the liver in cats. Acta physiol. scand. 1974. 91 42 52.

rup N J A Larsen and A Munck, Imitation of glucagon effects on splanchnic hemodynamics nd liver function by N⁶ 2'-O-dibutyryl 3',5'-cyclic AMP (DBcAMP) in cats. Acta physiol. scand. 1975. 5 110–116.

tz, J U Peiper and E Bauereisen, Appearance and site of venovenomotoric reactions in the liver rculation. Pflügers Arch. ges. physiol. 1968. 299 311–25.

tner, H and E P Pick, Über die durch "Schockgifte" erzeugten Zirkulationsstörungen. Münch. med Wschr 1915. 34 1141–43

oltholt J and T Simmann, Die Wirkung von Acetylcholin, Bradykinin und Angiotensin auf die urchblutung der Leber des narkotisierten Hundes und auf den endexhalatorischen Druck im Ductus chole- dochus. Pflügers Arch. ges. physiol 1968. 300. 189–201.

holz, R., Untersuchungen zur Redoxkompartimentierung bei der hämoglobinfrei perfundierten Ratten- leber In: Stoffwechsel der isoliert perfundierten Leber Ed. Staib, W and R. Scholz, Springer Verlag Berlin-Heidelberg. 1968 p 24–47

a, Y J R. C Mott and A. J. Watt, Vasopressin effect on portal and systemic hemodynamics JAMA 1969. 216 1065–68.

reiler, K., S Keiding and N Tygstrup Clearance as quantitative measure of liver function. In The Liver quantitative aspects of structure and function. Ed. Paumgartner, G., Karger Basel. 1973.

during histamine administration This cannot be explained by a baroreceptor r response to the decreased arterial blood pressure, as the hepatic nerves surround hepatic artery and portal vein had been cut to secure contact between the flow pro the vessels. The constriction may be explained by a direct effect of histamine on the arterioles. Also, an increase in sinusoidal pressure—reflected by the increase in pressure—may elicit constriction of the hepatic arterioles (Lutz, Peiper and Bh 1968) The lack of effect of histamine on the conductance in the intrahepatic low-p vessels indicates that a histamine-sensitive hepatic venous sphincter does not exist in contrast to dogs (Mautner and Pick 1915) The decrease in gastrointestinal cond during vasopressin infusion is a well documented finding and the resultant decrease in pressure has been used in the treatment of hemorrhage from oesophageal varices. A p direct constrictor effect of vasopressin on the hepatic arterioles may be masked by a di as a response to the decrease in sinusoidal pressure, in accordance with the findir Cohen *et al* (1970). In the study of Cohen *et al* (1970) the constrictor effect of angio on the hepatic artery was less pronounced than in the present experiments. This discrep may be due to the relatively unaffected portal—and hence sinusoidal—pressure in the ent study The decreased portal venous conductance during angiotensin infusion is p ably due to constriction of the intra-hepatic portal ramifications (Scholtholt and Sh 1968)

According to the model for ICG elimination proposed by Winkler Keiding and Tyg (1973) the decrease in ICG clearance during the infusions of vasopressin may be expl by the decrease in EHBF with an unaltered function of the ICG eliminating system. In other experiments where EHBF was not significantly changed, no effect on the ICG cl ance was found. The small decrease in bile flow is probably due to a depression of ductular bile formation, since the excretion of ICG was unaffected and the excretion of bicarbonate decreased in proportion to the bile flow Judged from the ethanol elim tion, liver function was unaffected despite the significant changes in the distribution hepatic blood flow on the portal vein and hepatic artery and/or the changes in hep arterial and portal venous conductances. The maintained functional capacity of the l indicates that the number of functioning liver cells and thereby the number of sinus perfused was not affected by the drugs. For vasopressin the results agree with finding humans, where infusion of vasopressin did not change the galactose elimination rate, a parameter reflecting the "functional liver mass" (Jacobsen, Ranek and Tygstrup 1969)

The unaltered hepatic oxygen consumption also reflects that gross uneven perfusion sinusoids is not elicited by the drug infusions. The increase in the lactate concentration during infusion of histamine must be due to peripheral glycolysis, as the splanchnic lac output was not affected Neither did angiotensin influence the splanchnic lactate outp During the vasopressin infusions, a rise in the splanchnic lactate output was due to lact production in the prehepatic splanchnic area. Hypoxia in even minor parts of the sinus would be expected to increase the hepatic lactate production and the HB/Ac ratio in hep venous blood (reflecting the mitochondrial redox level (Scholz 1968), but such alteratio were not observed. The gastro-intestinal lactate production during vasopressin infu corresponds to the findings of Silva, Moffat and Watt (1968) and may be explained

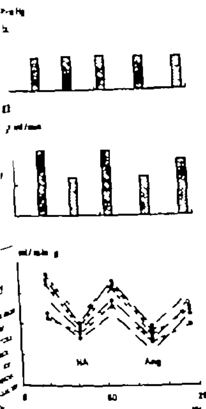


Fig. 1 Effect of noradrenaline (NA) and angiotensin (Ang) infusion on arterial pressure ($\bar{A}P$), total renal blood flow (RBF) and local cortical blood flow (CBF) at 6 different electrode sites in one dog. Open circles: Inner cortex. Closed circles: Outer cortex.

Methods

effect of adrenaline (A), noradrenaline (NA), angiotensin (Ang) (Hypertensin, CIBA) and vasopressin OH) (Parmenn, Parke, Davis & Co.) on renal blood flow was determined in 9 expts. on 6 mongrel dogs both sexes, weighing 15–21 kg. The animals had free access to water and food until 12 h prior to the periments. The food consisted of fish and pellets providing about 5 g NaCl/24 h. Anesthesia was induced i.v. injection of Nembutal, 25 mg/kg b.wt. Additional doses of 5 mg/kg were given when needed. Polyethylene catheters were placed in brachial vein, brachial and saphenous artery for infusions d arterial pressure (AP) recording.

The kidney was exposed retroperitoneally through flank incision. An electromagnetic flowmeter probe (Nycotron) was placed on the renal artery for continuous measurement of total renal blood flow (RBF). Calibration of the flowmeter was performed on the femoral artery. A polyvinyl catheter was inserted in the renal artery with the tip directed upstream. A Blalock clamp was placed on the aorta above the renal arteries to control the renal arterial pressure during the infusion of vasoconstrictor agents.

Three L-shaped platinum electrodes were placed in the outer cortex with the sensitive electrode tips 4 mm deep, and 3 other electrodes 5–8 mm deep in the inner cortex. The shafts of the electrodes were fixed to the renal capsule by 2 sutures and the electrodes carefully covered with perirenal fat and the wound closed by towel clips.

Hydrogen concentration in the tissue around the sensitive electrode tip was determined polarographically at a polarization potential of +0.2 V versus an Ag/AgCl electrode placed subcutaneously on the hip (Aukland et al. 1973). The amplified electrode current was recorded on a 6-channel recorder (Rikadenki Kogyo Co., Model B-64). 3–10 ml saline saturated with hydrogen at 37°C was injected in the renal artery

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Effect of Vasoactive Agents on the Distribution of Renal Cortical Blood Flow in Dogs

By

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Abstract

TYSSEBOTN, I. and A. KIRKEBO. *Effect of vasoactive agents on the distribution of renal blood flow in dogs* Acta physiol. scand 1975 95 318–228

The distribution of renal cortical blood flow was studied in 6 Nembutal anesthetized dogs dur periods and during infusions of adrenaline, noradrenaline, angiotensin and vasopressin. Loc blood flow was measured as H_2 gas desaturation rate recorded polarographically by platinum in outer and inner cortex. The total renal blood flow (RBF) was measured by an electromagn meter In the control period the outer cortical blood flow (OCF) and inner cortical blood fl averaged 3.59 (± S.D 0.85) ml/min g and 3.23 (± S.D. 0.64) ml/min g, respectively Infusions of th vasoactive agents caused essentially equal vascular responses. All agents caused increased local re ance and reduction of RBF whether given intravenously or intraarterially The RBF could be to 20–50 % of initial control flow by increasing doses of vasoactive agents. OCF and ICF fell propor and almost to the same extent as RBF or OCF fell slightly more than ICF There was no ev patchy o zonal hypoperfusion in cortex caused by infusion of adrenaline, noradrenaline, angiot vasopressin.

It is well known that adrenaline, noradrenaline, angiotensin and vasopressin incr vascular resistance in the kidney and decrease total renal blood flow (RBF). H several investigators have claimed that the resistance is not increased in all parts kidney Thus Trueta *et al* (1947) found in rabbits that large doses of adrenaline an pressin reduced the amount of Indian ink and angiographic contrast medium reach outer cortex, whereas more contrast was found in the juxtamedullary circulation recently Carriere (1969) and Grandchamp, Ayer and Truniger (1971), using external ing of ^{133}Xe or ^{85}Kr washout, reported that vasopressor infusion caused a marked re of flow in outer cortex, whereas flow in the juxtamedullary cortex and outer medulla re unchanged or even increased However this evidence of a preferential vasoconstriction outer cortex has not been confirmed by measurements of microsphere distribution (*et al* 1972) and local heat clearance (Grängsjo and Persson 1971). In view of these con results we have reinvestigated the question of the distribution of blood flow in the during the renal vasoconstriction produced by several vasoactive agents, using washout method which permits repeatable local blood flow measurements.

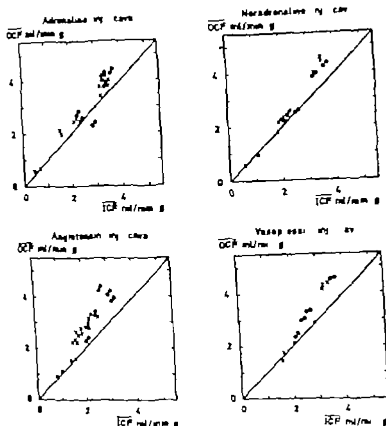


Fig. 2. Mean blood flow in outer cortex ($\overline{OCF}$) related to mean flow in inner cortex ($\overline{ICF}$) during i. ... ion of the vasoactive agents. Closed circles: Control period. Crosses: Test period.

...9) ml/min g. Mean arterial pressure in control periods averaged 121 (range 100–145) ...m Hg.

RBF and local blood flow fell progressively with increasing doses of all vasopressor ...nts.

There was a proportional reduction of OCF and ICF or a slightly greater fall in OCF ...n ICF for each of the four vasopressor agents: A, NA, Ang and ADH when infused ...to the renal artery (Fig. 2–6). The ratio $\overline{OCF}/\overline{ICF}$ between mean flow of the three electrodes ...outer cortex ($\overline{OCF}$) to mean flow of the three electrodes in inner cortex ($\overline{ICF}$) in different ...periments was 1.05–1.23 in control (not significantly higher than unity), and fell to 0.92–...14 during infusion of the four vasoconstrictor agents (Table I). The small decreases in ...e OCF/ICF ratio, 5–13 % below control, were statistically significant for i.a. infusion of ...A, NA and Ang.

For all agents the mean ratio $\overline{OCF}/\overline{ICF}$ calculated from local flow values in per cent of ...ntrol, were insignificantly lower for flow values smaller than 67 % than for flow values ...etween 67–100 % of control.

TABLE I. OCF/ICF ratio (S.E.) in control periods and during infusion of adrenaline (A), no (NA), angiotensin (Ang) and vasopressin (ADH).

Agents	Control	Arterial infusion	p, paired t test	Control	Venous infusion	p H
A	1 18 ± 0.09	1.07 ± 0.05	< 0.05	1 10 ± 0.06	1.03 ± 0 05	
n	29	34		12	17	
NA	1 05 ± 0.07	0.92 ± 0.06	ns	1 10 ± 0.03	1.09 ± 0.04	n
n	20	29		10	15	
Ang	1.‥3 ± 0.07	1 14 ± 0.03	< 0.05	1.24 ± 0.04	1.29 ± 0.05	n
n	8	16		10	20	
ADH	1 14 ± 0.07	1.09 ± 0.06	ns	1.26 ± 0.04	1 13 ± 0 06	n
n	8	10		10	12	

p, calculated by paired t-test pairing one or two test ratios to the last control ratio measu min before infusion periods. (All differences were nonsignificant using unpaired t-test.)

n is the number of infusion periods.

ns, nonsignificant.

through the polyvinyl catheter until constant H_2-concentration was obtained at the differ sites. The injection was then suddenly stopped and the washout curves were recorded, a plotted on semilogarithmic paper Blood flow was determined from the formula 0.69λ/T where $T_{1/2}$ is the half life of the hydrogen concentration in the tissue.

The vasoactive agents were dissolved in 0.9 % saline and infused at a rate of 0.1 2.5 ml/m artery by a syringe pump (Sage Instrument, Mod. 355) or i by a peristaltic pump (Multi 0.2 15 ml/min. The concentration of A, NA, Ang and ADH in the infused solution were 2 µg 10 µg/ml and 0.1 PU/ml respectively

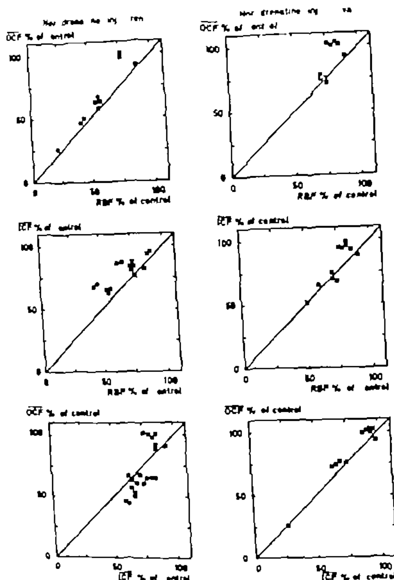
The local flow was determined at several infusion rates, reducing blood flow to all levels be and the lowest RBF which we could keep stable within 3 % of control flow Usually this level was 10–25 % of control. The determination of the hydrogen washout rate as al ys constant RBF obtained by careful djustment of the infusion rate of vasoactive agents in the Under i.v infusion of large doses of vasoactive gents a rise in renal arterial pressure was ke mm Hg by tightening the Blalock clamp on the rta. In 11 v infusion periods the perfusion lowered to 50–70 mm Hg by the Blalock clamp

At the end of each experiment the kidney was excised drained and weighed. The location of tips in cortex were carefully examined. Using Student paired t test, differences were consider at $p < 0.05$.

Results

Infusions were usually sustained for 3 to 15 min before flow measurements. Rep sions of different vasoactive agents were given with intervals of recovery be infusion as shown in Fig. 1 New infusion of a vasopressor agent was not m RBF and local cortical flow had increased close to control level.

Renal arterial infusion of vasopressor agents Control measurements of RBF det electromagnetic flowmeter varied between 3 20 and 6.40 ml/min g kidney weight dogs with an average of 4.20 (± S.D 1.20) ml/min g. The local flow measured in c of individual dogs ranged from 2.17 to 4 64 ml/min g with an average of 3. 0.85) ml/min g and flow in inner cortex from 2.33 to 4.23 with an average of 3.



ig. 4 The effect of noradrenaline infused into the renal artery and the vena cava on the total renal blood ow (RBF) and the local cortical blood flow (OCF ICF). Open circles. Renal arterial pressure lowered y aorta clamp

OCF) is plotted against the mean flow of 3 electrodes in the inner cortex (ICF) in the different xperiments.

The OCF/ICF ratios in control measurements and during i.v. infusions of NA, Ang and ADH were 1 10 to 1.26, all significantly higher than unity No significant change of the ratio was obtained during administration of NA, Ang and ADH (Table I). i.v. infusion of A caused small, but statistically significant reduction in ratio, as did i.a. infusion.

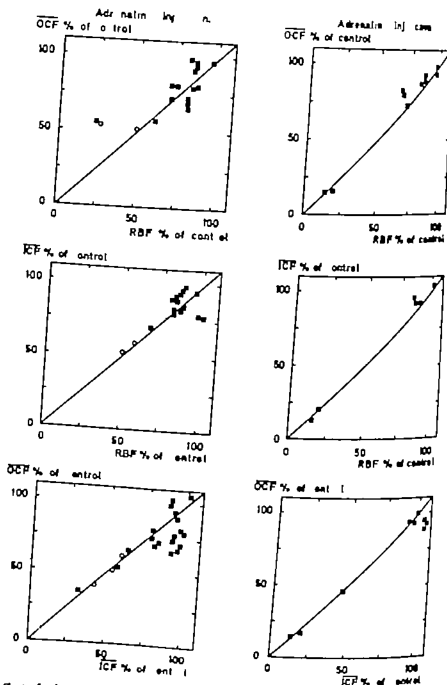


Fig. 3 The effect of adrenaline infused into the renal artery and the vena cava on the total renal bl… flow (RBF) and the local cortical blood flow (OCF ICF). Open circles. Renal arterial pressure lowe… by aortic clamp.

Intravenous infusion of vasopressor agents The last control OCF and ICF prior to i… infusion of the different agents averaged 3.37 (±S.D 0.90) ml/min g and 2.85 (±S.D. 0.… ml/min g. Control RBF and $\bar{A}P$ were 3 70 (S D 0 87) ml/min g and 109 mm Hg, respectively

Intravenous infusion of the vasoactive agents caused similar flow changes as describ… above for intraarterial infusion. In Fig. 2 the mean flow of the 3 electrodes in the outer cort…

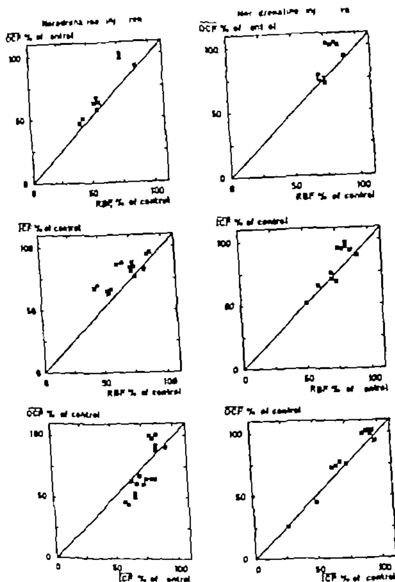


Fig. 4. The effect of noradrenaline infused into the renal artery and the vena cava on the total renal blood flow (RBF) and the local cortical blood flow (OCF ICF). Open circles: Renal arterial pressure lowered by aorta clamp.

OCF) is plotted against the mean flow of 3 electrodes in the inner cortex (ICF) in the different experiments.

The OCF/ICF ratios in control measurements and during i.v. infusions of NA, Ang and ADH were 1.10 to 1.26, all significantly higher than unity. No significant change of the ratio was obtained during administration of NA, Ang and ADH (Table I). I.v. infusion of A caused small, but statistically significant reduction in ratio, as did i.a. infusion.

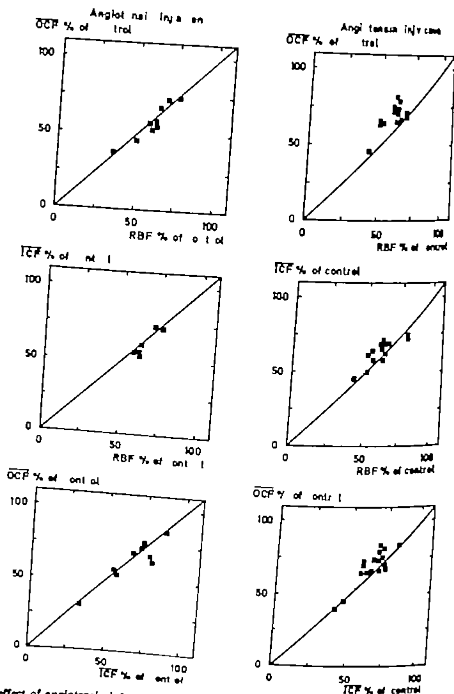


Fig. 5. The effect of angiotensin infused into the renal artery and the ena ca on the total renal blo flow (RBF) and the local cortical blood flow (OCF ICF) Open circles. Renal arterial pressure lowe by aortic clamp.

The local control flow showed great variation between individuals. The results were therefore recalculated as percentage of the control flow to make values more comparable (Fig. 3–6). Typically flow at single electrode sites showed reductions essentially similar to the reduction of mean flow

Whereas RBF could be completely stopped by high doses of NA, we were not able to

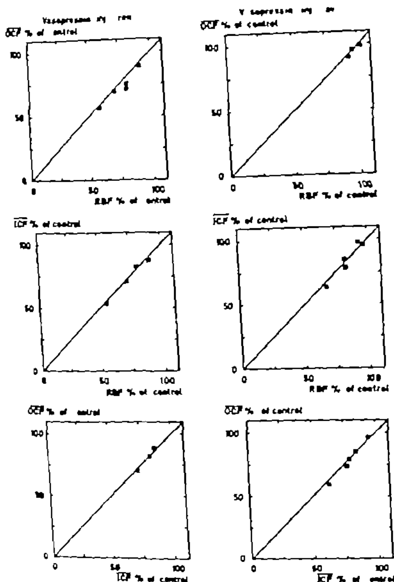


Fig. 6 The effect of vasopressin infused into the renal artery and the vena cava on the total renal blood flow (RBF) and the local cortical blood flow ($\overline{OCF}$ $\overline{ICF}$).

maintain a constant RBF of less than 25% of control. I.v. infusions of NA and Ang caused a marked raise in $\overline{AP}$ but renal arterial pressure was held constant by adjustment of the aortic clamp.

The lowest RBF obtained by infusion of A, Ang and ADH were respectively 20%, 20% and 45% of control.

Discussion

Flow per gram tissue measured with the microsphere method shows higher valu outer cortex and lower in the inner cortex (Rector *et al* 1972) than those obtain present and earlier studies using the H clearance method (Aukland *et al* 1971, and Tyssebotn 1974) This disagreement may be more apparent than real. The mic method measures glomerular flow and since flow per glomerulus is not very dif the various layers of the cortex (Horster Kemler and Valtin 1971) more microsph necessarily be found in the outer cortex. On the other hand, washout of a diffusible such as H_2 must be determined mainly by flow in the peritubular capillaries becaus much greater length and surface area compared to the glomerular capillaries. Si tubules in outer and inner cortex have much the same transport functions, ther good reason to expect a great difference in peritubular blood flow in the two zones.

The main finding in the present study was a fairly proportional flow reduction i and inner cortex with all the vasoconstrictors tested, indicating an evenly distributed constriction. The slightly greater fall in OCF than in ICF can hardly be of any physi importance. Since cortical blood flow constitutes at least 70%, and probably closer t of total renal blood flow variations in average cortical flow should closely parallel ch in total renal blood flow (Barger and Herd 1973) This requirement is satisfied in the p findings (Fig. 3–6), thus providing indirect evidence for the validity of the H metho measuring total cortical flow

While no measurements were made in the medulla in the present study the proport reduction of RBF and OCF and ICF with all 4 vasoconstrictors would suggest a proport reduction also of medullary flow However because of the small flow fraction of the me this reduction is liable to great errors, but agrees well with a proportional reduction of and H_2-clearance in outer medulla reported by Aukland (1968). Similarly Grångsjo and son (1971) found reduction of both cortical and medullary flow during infusion A, ADH and high doses of NA but the heat clearance method used did not permit any q titative estimate of absolute or relative changes in blood flow A qualitatively diffe result for which we have no explanation, was their finding of unaltered or even incre flow in medulla during i v infusion of small doses of NA.

While microsphere distribution differs from the present results during control conditio as discussed above, Rector *et al* (1972) found equal relative reduction of microsph uptake during infusion of NA and Ang in outer and inner cortex in good agreement with t present results.

As under several other experimental conditions completely different conclusions ha been drawn from externally recorded washout of ^{85}Kr or ^{133}Xe and subsequent compa mental analysis During infusion of NA (Carriere 1969 Grandchamp, Ayer and Truni 1971) the renal volume fraction corresponding to component I — assumed to repres cortex under control conditions, — is markedly reduced while the rate constant, indicati flow per gram tissue is unchanged or slightly reduced. Component II normally conside to represent inner cortex and outer medulla usually shows increased flow rate and the volume. Obviously the anatomical counterparts to these components change during va

rictions, and the interpretation given is that flow is reduced in outer cortex to such an t that it becomes equal to an increased outer medullary flow thus accounting for the ...rise in the volume corresponding to component II. Flow in inner cortex is not much ...ged and is represented by the now less voluminous component I. While this interpreta... was supported by autoradiograms, no quantitative measurement of local gas concentra... was presented to validate the conclusions.

...ing washout of ^{133}Xe Grandchamp *et al.* (1971) found a similar redistribution of blood ... during infusion of Ang (+Dibenzyline) whereas Carriere and Friborg (1969) found a ...ase in both cortical and medullary blood *flow most pronounced in the subcapsular* ...merular region of the cortex. From the autoradiograms, they concluded that component ... the washout curve, believed to correspond to outer medulla under normal condition, ...sents regions of the cortex with a slower blood flow rate during Ang infusion. The ...out of ^{85}Kr was slower in outer medulla than in any part of the cortex.

...e marked discrepancy between ^{133}Xe or ^{85}Kr washout and *measurements of flow* at ...eptable anatomical locations obtained with H washout and other local recordings ...ests to us that external recording of ^{133}Xe or ^{85}Kr washout does not provide valid inform...n on intrarenal blood flow distribution.

...f more interest is the autoradiographic demonstration of reduced uptake of ^{85}Kr in the ...ow subcapsular zone during Ang infusion (*Carriere and Friborg 1969). Since in the* ...ent study the electrodes in outer cortex were usually placed 1 3 mm underneath the ...al surface, we cannot exclude a more pronounced vasoconstriction in most superficial ...tical layer On the other hand, we found no evidence for a patchy or segmental cortical ...emia as indicated by white areas in ^{85}Kr-autoradiograms (Carriere 1969 Grandchamp *...al. 1971). The implications of the present results for renal excretory functions are not* ...ious. It has been theorized that reduction of salt excretion usually obtained during ...sion of vasoconstrictor agents should be due to a redistribution of glomerular filtrate ...m superficial "salt loosing nephrons to deep "salt retaining" nephrons (Pomeranz, ...ich and Barger 1968). However no convincing experimental evidence for the existence ... two such *functionally different nephron populations has* been presented, and the present ...dy would rather suggest that salt retention should result from an even reduction of deep ...d superficial filtration rate and/or reduced peritubular capillary pressure.

The largest doses of vasoconstrictors used probably greatly exceed maximal endogenous ...oduction of these substances. However, combined effect of raised plasma concentrations ... all these substances such as obtained, *for instance during hemorrhagic* hypotension ...cormick and Paladin 1964 Watts and Westfall 1964) might well be responsible for a ...ubling of *renal* vascular resistance. The finding of uniform distribution of blood flow ...uring hemorrhagic hypotension (Aukland *et al* 1973 Kirkebø and Tyssebotn 1974) is ...erefore well consistent with the present results.

References

...UKLAND, K., Effect of adrenaline, noradrenaline, angiotensin and renal nerve stimulation on intrarenal distribution of blood flow in dogs. *Acta physiol. scand.* 1968. 72. 498–509

...UKLAND, K., A. KIRKEBØ, E. LØYNING and I. TYSSEBOTN, Effect of hemorrhagic hypotension on the distribution of renal cortical blood flow in anesthetized dogs. *Acta physiol. scand.* 1973. 87 514–525.

Barger, A. C. and J. A. Herd, Renal vascular anatomy and distribution of blood flow. In Orlof, Berliner and S. R. Geiger (Eds.), *Handbook of Physiology* Section 8: Renal Physiology. Am Soc. Wash. 1973 269–313.

Carrière, S., Effect of norepinephrine, isoproterenol, and adrenergic blockers upon the intra bution of blood flow *Canad. J. Physiol. Pharmacol.* 1969 47 199–208.

Carrière, S. and J. Friborg, Intrarenal blood flow and PAH extraction during angiotens *Amer. J. Physiol.* 1969 217 1708–1715.

Grandchamp, A., G. Ayer and B. Truniger, Pathogenesis of redistribution of intrarenal blo hemorrhagic hypotension. *Europ. J. clin. Invest.* 1971 1 271–276.

Grängsjö, G. and E. Persson, Influence of some vaso-active substances on regional blood flo kidney *Acta anaesth. scand.* 1971 15 71–95.

Horster, M., B. J. Kemler and H. Valtin, Intracortical distribution of number and volume of during postnatal maturation in the dog. *J. Clin. Invest.* 1971 50 796–800.

Kirkebø, A. and I. Tyssebotn, Distribution of renal cortical blood flow during hemorrhagic h in conscious dogs. *Acta physiol. scand.* 1974, 91 22–31.

Pomeranz, B. H., A. G. Birtch and A. C. Barger, Neural control of intrarenal blood flow *Physiol.* 1968. 215 1067–1081.

Rector, J. B., J. H. Stein, W. H. Bay, R. W. Osgood and T. F. Ferris, Effect of hemorrhage pressor agents on distribution of renal blood flow *Amer. J. Physiol.* 1972. 222. 1125–1131.

Scornik, O. A. and A. C. Paladini, Angiotensin blood levels in hemorrhagic hypoten related conditions. *Amer. J. Physiol.* 1964 206 553–556.

Trueta, R. J., A. E. Barclay, P. M. Daniel, K. J. Franklin and M. M. L. Prichard, Studies circulation. Blackwell Scientific Publ. Ltd. Oxford 1947.

Watts, O. T. and V. Westfall, Studies on peripheral blood catecholamine levels during shock in dogs. *Proc. Soc. exp. Biol. (N.Y.)* 1964 115 601–604.

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Time Courses of Pulmonary Gas Exchange and Heart Rate Changes in Supine Exercise

By

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Abstract

KARLSSON, H., B. LINDBORG and D. LINNARSSON. *Time courses of pulmonary gas exchange and heart rate changes in supine exercise.* Acta physiol. scand. 1975. 95. 329–340.

The time courses of ventilation ($\dot{V}_E$), O_2 uptake ($\dot{V}_{O_2}$), CO_2 elimination ($\dot{V}_{CO_2}$), respiratory exchange ratio (R), end-tidal P_{O_2} and P_{CO_2} and heart rate (HR) were studied in seven subjects performing light dynamic leg exercise in the supine position. Individual and group mean time courses in response to step changes in work load were computed and displayed graphically. A computer-based method was also used to fit mono- or bi-exponential mathematical functions to the recorded responses. The over-all rate of HR change in response to the transition from 0-load pedalling to exercise (on-response) was faster (mean response time, MRT = 31 s) than the corresponding $\dot{V}_{O_2}$ response (MRT = 45 s) while $\dot{V}_E$ responded considerably slower (MRT = 86 s). During the reverse transition (off-response), $\dot{V}_{O_2}$ and $\dot{V}_E$ changed with the same rate as in the on-response, while the HR-change was slower than during the on-response (MRT = 50 s). During the initial 15-sec period, $\dot{V}_{O_2}$ changed only slightly. Such contrasts to previous results in the sitting position, where 50 % of the final change in $\dot{V}_{O_2}$ has been reported to occur within the first 15-sec period, and where changes in blood distribution and stroke volume are known to be more pronounced than in the supine position. Our results emphasize the importance of central circulatory changes for the time course of $\dot{V}_{O_2}$ at the start and end of exercise.

The time course of oxygen uptake ($\dot{V}_{O_2}$) at the start of exercise can be considered to be the net result of a multitude of adaptive changes of respiratory, circulatory and metabolic nature. Auchincloss, Gilbert and Baule (1966) showed that variations in the response pattern of ventilation influenced the time course of gross $\dot{V}_{O_2}$, but did not greatly alter the time course of the pulmonary-capillary O_2 uptake, which was suggested to be mainly influenced by the dynamics of circulatory changes. In a recent study Linnarsson (1974) showed that two phases could be discerned in the time course of $\dot{V}_{O_2}$ at the start of light exercise in the sitting position, a) a rapid initial change, assumed to result from an equally rapid readjustment of the blood flow through the lungs, b) a second rapid change after about 15 s, assumed to reflect the appearance of blood from the working muscles in the lungs. Each of the two phases constituted about 50 per cent of the final $\dot{V}_{O_2}$ change. In the supine position, however, when changes in stroke volume, cardiac output and blood distribution are less marked (Bevegård, Holmgren and Jonsson 1960, Jones *et al.* 1970, Raynaud *et al.* 1973) the time courses of pulmonary gas exchange may differ from those at the onset of sitting exercise. It was therefore thought of interest to experimentally determine the time courses of pulmonary gas

TABLE I. Anthropometric data of subjects and work loads.

Subject	Age years	Weight kg	Height cm	Work load Watt
BL	24	64	176	107
TH	24	64	179	107
DL	29	61	172	98
BS	1	64	170	82
HK	27	61	176	82
MN	27	65	179	98
LF	32	81	189	107

exchange and heart rate changes in response to light to moderate exercise in the supine position

Experimental and computational methods

Subjects. Seven healthy male sedentary subjects participated in the study For individual dimensions see Table I

Equipment The subjects exercised on an electrically braked cycle ergometer (Elema, Sweden) adapted for supine leg exercise (for details, see Wigertz 1971). The work load could be automatically switched from "zero load" (pedalling with no work load) to a preset work load at predetermined time intervals. Heart rate (HR) was obtained from chest electrodes and a linear beat to-beat cardiotachometer (Linnarsson, Wigertz and Ödman 1969). The subjects breathed humidified room air through a respiratory valve, the expiratory outlet of the valve being connected to a Venturi-type flowmeter (Wigertz 1969). A sample of the respired gas in the mouthpiece was drawn at a rate of 1 l/min through fast-responding O_2 (Westinghouse model 209) and CO_2 (Capnograph) analyzers. The outputs of the flow and gas concentration transducers were fed into an analog computer (PACE TR-48), where pulmonary ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}o_2$), carbon dioxide elimination ($\dot{V}co_2$) and respiratory exchange ratio (R) were computed breath by breath (Linnarsson and Lindborg 1974). These variables, together with HR and end-tidal concentrations of O_2 and CO_2 ($P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$ respectively) for each expiration obtained with an analog peak-holding device (Carlsson *et al* 1965), were displayed on an eight-channel analog ink-pen writer (Mark 200) and stored on a 14-channel FM tape recorder (Ampex FR 100) for subsequent off-line processing of data. A flow chart showing the collection and the processing of data is given in Fig. 1

Experimental procedure The subjects performed supine exercise at a work load corresponding to approximately 45 per cent of maximal aerobic capacity (Table I) in 6-min periods alternating with 10 min zero-load pedalling (pedalling at 60 rpm with no work load imposed). Each experiment started with a 6-min warm-up period followed by four 10-min zero load—6-min exercise periods, and measurements thus could be made during four *off* transients (work to zero-load transient) and four *on*-transients (zero load to work transient). In order to avoid influence of anticipation on the measured responses, the load was changed without the subject's prior knowledge.

Data processing The off-line data processing (Fig. 1) was performed by means of 2 digital computers. A computer with data acquisition capabilities (IBM 1800) was used for analog/digital conversion, calibration, correction and averaging of the recorded responses. The model estimation and data presentation procedures were performed using a large computer (IBM 360/75).

Seven channels were transferred from the tape recorder to the computer: work load, HR, $\dot{V}_E$, $\dot{V}o_2$ (uncorrected), $\dot{V}co_2$, $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$. The sampling interval was 1 s. True R and true $\dot{V}o_2$ were computed for each sampling interval from $\dot{V}co_2$ and uncorrected $\dot{V}o_2$ using the following relationships to correct errors in $\dot{V}o_2$ due to V_I/V_E inequalities, when $R \neq 1.0$

If

$$\text{measured R} = \frac{\dot{V}co_2}{\text{measured } \dot{V}o_2}$$

then

$$\text{true R} = \frac{(1 - F_{I_{O_2}}) \text{ measured R}}{1 - F_{I_{O_2}} \text{ measured R}} \quad \text{(Linnarsson and Lindborg 1974)}$$

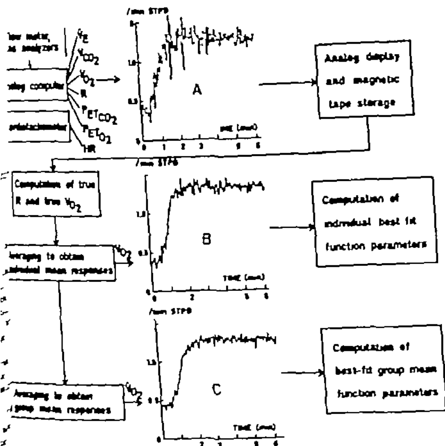


Fig. 1. Schematic diagram showing the analog and digital computer operations to compute, correct, average, display and parameterize the time course of V_{O_2}. Curve A is a recording of a single on-response of V_{O_2}, B the average of 4 responses in the same subject, and curve C shows the group-mean response of subjects.

$$\text{true } V_{O_2} = \frac{V_{CO_2}}{\text{true R}}$$

For each subject and variable the 4 on- and the 4 off-responses were synchronized to the work load changes and averaged, so that each consecutive sample of the individual mean response was computed as the average of 4 corresponding values of the recorded responses with identical time-relationships to the work-load change. This procedure reduces the level (S.D.) of stochastic variations by a factor of 2, thus enhancing the basic time pattern of the response.

To remove short-lasting technical artefacts due to e.g. tape recorder drop-outs, an automatic artefact elimination procedure was applied to the individual mean responses (Bremen, Wigertz and Nygårds 1970). Each sample of the response was compared with the average of the values in a window symmetric around the sample, where a specified number of the smallest and the largest values were removed. If the difference between the sample and the average was greater than a preset limit value the sample was replaced by the average. The window width, number of removed values, and difference limit value were individually set for each variable, dependent on response and disturbance characteristics.

To obtain the dynamic response characteristics for the entire group of seven subjects, group mean responses were computed using the same procedure as that used to obtain individual mean responses. The effect of this averaging is a reduction of the level (SD) of the stochastic variations by a factor of $\sqrt{7}$ but the characteristic response patterns are smoothed if they occur time scattered in relation to the work load change.

To facilitate comparison between the responses of the different variables we attempted to find simple parametric descriptions of the transients. Since some of the variables under study have been described to behave like exponential changes (*i.e.* Margaria, Edwards and Dill 1933, Cerretelli, Sikand and Farhi 1966) mono- and bi-exponentially decaying functions were fitted to the responses. The functions used were of the form:

$$f_1(t)\begin{cases}0 & \text{for } t<T_D\\ a_1[1-\exp(-(t-T_D)/\tau_1)] & \text{for } t>T_D\end{cases}$$

$$f_2(t)\begin{cases}0 & \text{for } t<T_D\\ a_1[1-\exp(-(t-T_D)/\tau_1)]+a_2[1-\exp(-(t-T_D)/\tau_2)] & \text{for } t>T_D\end{cases}$$

where a_1 and a_2 are amplitude coefficients, τ_1 and τ_2 time constants and T_D is time delay, which in the bi-exponential case is common to both time constants. The same fitting functions have been used earlier by Broman and Wigertz (1971) and Linnarsson (1974). The parameters were estimated by means of the least squares method, *i.e.* they were chosen to minimize the sum of squared differences between the function and the samples of the recorded response (loss function, L). The variance of the differences can be estimated by $S^2 = L/(N-n)$, where N is the number of samples and n the number of estimated parameters. The fitting interval lasted from approximately 30 s before the change in work load until the next shift in work load. Since $f_1(t)$ and $f_2(t)$ are nonlinear with respect to time delay and time constants, an iterative procedure was required to minimize L. Several minimization procedures were tried and the method developed by Marquardt (1963) was found to be the most suitable.

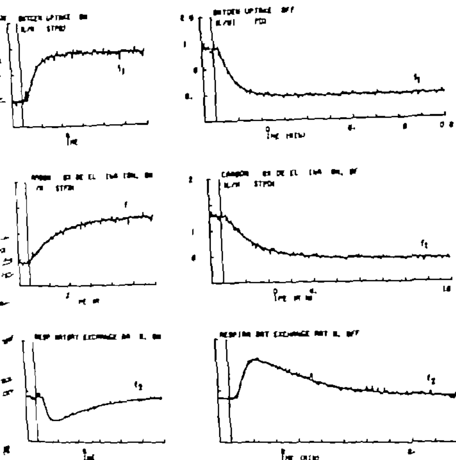
The mean response time (MRT) can be used to indicate how fast a variable responds to a sudden change in the input function. For a monoexponential response, MRT is the sum of the time delay and the time constant. If the deficit value of a step response is defined as the area between the response and the steady state end value from the moment of work load change until steady state is reached, this value is equal to the response amplitude multiplied by the mean response time (*cf.* Whipp 1971, Linnarsson 1974).

Results

The group mean responses of V_E, V_{CO_2}, V_{O_2}, R, P_{ETCO_2}, P_{ETO_2} and HR to changes in work load from zero load to light exercise and in the reverse direction are depicted in Fig. 2 together with the best-fit functions of first or second order. Function parameters, *i.e.* best-fit values of amplitudes, time constants and time delays for the group-mean responses are presented in Table II together with mean values of the function parameters from individual responses.

Configuration of the individual and group-mean curves. Some typical response patterns consistently occurred in the individual responses of HR and V_{O_2}. The on-response of HR always displayed an initial steep increase with no delay, followed after 10–20 s by a levelling-off or a transient decrease of 5–10 s duration. Thereafter HR increased at a rate considerably slower than the initial response. No such "notch" in the HR response was observed in the off transient. A fast initial increase of small amplitude, sometimes with an overshoot, followed by a levelling-off period and a secondary rise, was observed also for V_{O_2} in all subjects. After the cessation of work V_{O_2} remained increased for about 15 s, before a rapid fall took place. These typical individual response patterns occurred sufficiently uniform in relation to the work load changes to appear also in the group-mean responses (Fig. 2, 4).

Steady state values. Group-mean values for the last 30 s period of each condition have been determined for all variables and these values have been included in the computation of the values for the on- and off responses.



t. 2. Graphical representation of group-mean responses of oxygen uptake, carbon dioxide elimination
l respiratory exchange ratio to light supine exercise. Responses from both the transition between load-
s pedalling and exercise (on) and the reverse transition (off) are shown. Each tracing computed from
ath-by-breath recordings from 23 exps. in 7 subjects. 0-time denotes the time for the change in work
d. Best-fit functions of first (f_1) or second order (f_2) are superimposed on the recorded responses.

$\dot{V}_{O_2}$ during zero-load pedalling amounted to 0.49 l/min STPD and the corresponding R was 92.2 beats/min. $\dot{V}$ averaged 13.0 l/min BTPS which, as judged from the R and P_{CO_2} values (0.95 and 36 mm Hg, respectively), may have been a slight hyperventilation. t the end of the exercise period $\dot{V}_{O_2}$ averaged 1.41 l/min yielding an average work efficiency f 0.30, which is in agreement with the findings of Whipp and Wasserman (1969), who also etermined work efficiency during light exercise with zero load as the baseline condition, but sed conventional Douglas-bag technique for the V_{O_2} determinations. HR during the last 0 s of exercise averaged 123.3 beats/min, whereas $\dot{V}$ amounted to 31 l/min BTPS. End-tidal was 5 mm Hg lower and end-tidal P_{CO_2} was 6 mm Hg higher than during zero-load edalling.

First-order exponential functions could be fitted to both the on- and the off-responses of

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where a and a_2 are amplitude coefficients, τ and τ time constants and T_D is time delay ... exponential case is common to both time constants. The same fitting functions have been used ... Broman and Wigertz (1971) and Linnarsson (1974). The parameters were estimated by means of ... squares method, i.e. they were chosen to minimize the sum of squared differences between the functi the samples of the recorded response (loss function, L). The variance of the differences can be ... by $S^2 = L/(N-n)$, where N is the number of samples and n the number of estimated paramete fitting interval lasted from approximately 30 s before the change in work load until the next shift ... load. Since f (t) and $f_2(t)$ are nonlinear with respect to time delay and time constants, an iterative p was required to minimize L. Several minimization procedures were tried and the method devel Marquardt (1963) was found to be the most suitable.

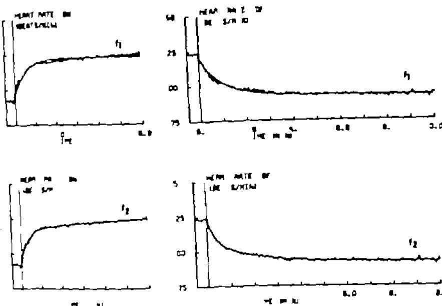
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Results

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Steady-state values. Group-mean values for the last 30 s period of each condition hav determined for all variables and these values have been included in the computation c values for the on- and off responses.



4 Group mean on- and off-responses of heart rate together with first- (upper tracing) and second-order (lower tracing) best-fit functions. The superior fitting of the second-order function is clearly shown. Symbols and conditions as in Fig. 2.

…n the fitted first-order functions. Thus both the on- and off-responses of HR were better described by second-order functions, as also is shown by the much lower S values in this …. This relationship is visualized in Fig. 4, where both first and second-order best-fit functions are graphically represented together with the measured responses.

Time delays. The time courses, described in terms of time constants and time delays, showed marked differences between the various variables. The general tendency was that variables with the slowest on-response also exhibited the slowest off-response, but except for $\dot{V}_{E}$, no symmetry between on- and off-responses was observed. Negative time delays were found in the best-fit first-order functions for V and HR, suggesting that the order of applied functions were too low since, when a second-order function was applied to HR, T_D approached zero (Table II). Positive time delays averaging between 12.5 and 22.8 s were found in the on- and off-responses of $\dot{V}_{O_2}$, R, $P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$ and in the off-responses of $\dot{V}_E$ and …

Time constants. The first-order time constants ranged from 28.6 s ($\dot{V}_{O_2}$ on) to 91.8 ($\dot{V}_E$ on). The second-order functions describing the biphasic responses of R and $P_{ET_{CO_2}}$ contained one short time constant ( ) and one 5–9 times longer time constant ( ) having opposite direction. In the second-order description of the mean HR responses, was approximately the same in the on- and off-transients, while differed markedly and was 4.5 times longer in the on-transient. This slow change, with −455 s and with an asymptote of 130.8 beats/min, may also be described as a linear change of HR averaging +1.2 beats min⁻¹ during the last … min of exercise.

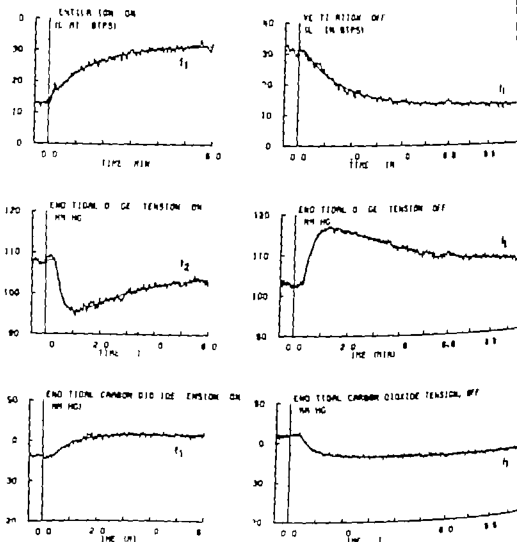


Fig. 3 Group mean on- and off-responses of ventilation, end tidal P_{O_2} and end-tidal P_{CO_2}. Symbols conditions as in Fig. 2.

V_E, $\dot{V}_{CO_2}$, $\dot{V}_{O_2}$ and $P_{ET_{CO_2}}$ both on an individual and on a group basis. Attempts to fit sec order functions to these 4 variables were made but no second time constants coul estimated. The values of the function parameters of the group-mean responses di approximated the mean values of individual parameters in the majority of cases. Gr averaging reduced the S value approximately in proportion to the number of subjects, i cating that the reduction of S obtained with the averaging procedure was accompli mainly through the reduction of stochastic variations.

Second-order functions were required to describe the biphasic on- and off-response pat of R and $P_{ET_{O_2}}$. The fitting procedure failed with individual data in one or more of subjects, so only the function parameters of the group-mean responses are given in Tabl

Both first and second-order functions could be fitted to the group-mean on- and responses of HR. Group averaging did not reduce the first-order S values in proportio the number of subjects, indicating that the experimental HR responses systematically diff

on- and off-responses (MRT appr. 45 s) did not differ significantly nor did oxygen ... (0.67 l STPD) differ significantly from oxygen debt (0.74 l STPD) (cf. above, "Data ...ssing").

Discussion

The main purpose of the present investigation was to study the interrelationships between the time courses of the O_2 uptake and the circulatory changes occurring during the unsteady ... of exercise. Oxygen uptake measured breath-by-breath at the mouth was used to assess the dynamics of the uptake of O_2 by the circulation in the lungs, and beat-by-beat heart-rate recordings were made to assess the corresponding adjustments of cardiac output.

In situations when large changes in pulmonary O_2 stores take place, resulting from changes in functional residual capacity (FRC) and/or in alveolar P_{O_2}, there will be a transient difference between V_{O_2} and the pulmonary-capillary O_2 uptake (Auchincloss, Gilbert and Baule 1966). During the first 30 s of light work in the sitting position, FRC decreases by about ... l (Linnarsson 1974) but no data seem to be available concerning FRC changes at the start and end of work in the supine position. The expiratory reserve volume (ERV) during supine rest is, however, substantially decreased compared to erect position (Sjöstrand 1951 ... et al. 1962) and it seems reasonable to assume, therefore, that only slight, if any, FRC changes take place at start and end of light supine work. Alveolar P_{O_2} is virtually unchanged during the initial 15–20 s period following the work-load change as reflected by the end-tidal values in the present study (Fig. 3, Table II) and by arterial measurements (...rstadt and Wigertz 1971). It can therefore be assumed that $V_{O_2}^{E}$ reflects with reasonable accuracy the pulmonary-capillary O_2 uptake during the initial 15–20 s of light supine exercise and recovery, while during the following 15–20 s period, when large changes in $P_{ET_{O_2}}$ take place, V_{O_2} will differ from pulmonary-capillary O_2 uptake. From the observed $P_{ET_{O_2}}$ changes this difference can be calculated to be some 0.1 l/min STPD representing a transient underestimation of pulmonary-capillary O_2 uptake at the start of work and a corresponding overestimation at the end of work.

It is well established that in the supine position the stroke volume during steady state of submaximal exercise does not differ greatly from resting values (Bevegård, Holmgren and Jonsson 1960, Jones et al. 1970). Raynaud et al. (1973) showed that this was true also for the unsteady state of exercise with the exception for the initial 30 s period after the onset of work, when the stroke volume exhibited a transient decrease by some 10 per cent. An opposite change of similar small magnitude has been observed during the first minutes of recovery from light supine exercise, when stroke volume has been reported to be increased by 10–20 per cent (Cumming 1972). Heart-rate changes can therefore be considered as fair estimates of the adjustments of cardiac output under the conditions of the present study.

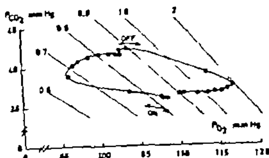
Over-all rates of response

Broman and Wigertz (1971), studying relatively well-trained subjects, found MRT values of about 15 s for the on-response of HR under conditions almost identical to that of the present study. The value of about 30 s for the sedentary subjects of the present study resembles the findings of Broman and Wigertz when using a much higher absolute work load, and this may indicate that the over-all rate of readjustment of HR is functionally coupled to the

TABLE II Parameterization of best-fit first and second-order functions for ventilation ($\dot{V}_E$, l/min BTPS), carbon dioxide elimination ($\dot{V}_{CO_2}$, l/min STPD), oxygen uptake ($\dot{V}_{O_2}$, l/min STPD), respiratory exchange ratio (R), end-tidal P_{CO_2} ($P_{ET_{CO_2}}$, mm Hg), end-tidal P_{O_2} ($P_{ET_{O_2}}$, mm Hg) and heart rate (HR, beats/min). Data refer to transients following onset of work (on) and recovery (off). Time constants (τ τ_2) and time delay (T_D) are given in sec. Group mean responses (upper value) as well as mean values ± standard error of the mean of the individual responses (lower values) are shown for each variable and parameter. Indicates that second-order models could be fitted to the group mean responses but not to all of the individual responses.

	a	τ	a_2	τ	T_D	[illegible]
on						
$\dot{V}_E$	18.8 19.3 ± 1.5	91.8 104.5 ± 26.1			−5.8 −4.7 ± 5.2	4.49 3.47
$\dot{V}_{CO_2}$	0.87 0.88 ± 0.06	84.2 86.0 ± 11.2			2.7 3.7 ± 4.9	0.77 5.29 [illegible]
$\dot{V}_{O_2}$	0.90 0.91 ± 0.03	28.6 29.3 ± 3.6			16.1 14.6 ± 3.8	1.54 7.66 [illegible]
R	−0.35	14.7	0.37	145.9	22.2	[illegible]
$P_{ET_{CO_2}}$	5.6 5.7 ± 0.7	45.9 43.6 ± 5.1			23.2 22.9 ± 4.8	[illegible] [illegible]
$P_{ET_{O_2}}$	−17.2	16.4	14.3	151.6	21.8	[illegible]
HR	30.3 30.5 ± 2.8	35.7 35.8 ± 4.9			−4.9 −4.6 ± 1.1	[illegible] [illegible]
HR	4.8	20.3	13.8	455	−1.7	[illegible]
off						
$\dot{V}_E$	−17.8 −17.8 ± 1.5	81.7 80.2 ± 5.2			12.5 14.6 ± 3.8	[illegible] [illegible]
$\dot{V}_{CO_2}$	−0.84 −0.84 ± 0.05	71.9 71.0 ± 4.5			12.5 13.6 ± 2.5	0.62 3.52 [illegible]
$\dot{V}_{O_2}$	−0.94 −0.93 ± 0.03	33.5 32.3 ± 1.5			14.1 15.3 ± 1.7	0.62 3.71 [illegible]
R	0.70	29.0	−0.69	133.1	21.0	0.112
$P_{ET_{CO_2}}$	−5.6 −5.7 ± 0.7	30.4 40.3 ± 16.6			22.8 21.4 ± 3.2	[illegible] [illegible]
$P_{ET_{O_2}}$	20.3	29.4	−15.9	172	20.0	[illegible]
HR	−30.3 −30.5 ± 2.7	56.6 56.6 ± 6.3			−6.2 −6.1 ± 1.7	[illegible] [illegible]
HR	−15.4	17.2	−15.7	101.3	0.8	[illegible]

The mean response time (see above "Data processing") represents the time for the "center of gravity" of the transient under study and can be used to compare the rates of adjustment of the various variables. $\dot{V}_E$ and $\dot{V}_{CO_2}$ were the slowest changing variables both in the on- and off-transients with MRT values between 85 and 95 s. The on-transients for HR represented the fastest change (MRT ≈ appr. 30 s) while its off-transient was 20 s slower ($p < 0.01$). F



... 5. O_2–CO_2-diagram showing the changes ... end-tidal P_{CO_2} versus end-tidal P_{O_2} during ... first 5 min of exercise (on) and recovery ... Filled circles denote start values and ... circles indicate every 30 s. The super-... R-isopleths show that end-tidal R ... generally were some 0.1 R units ... than the corresponding R values com-...ed from $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ (cf. Fig. 2).

...nt changes versus time delays in the initial responses of HR and $\dot{V}_{O_2}$

The immediate rapid HR increase at the onset of exercise was originally described by Krogh and Lindhard (1913) and it was thought to be associated with a rapid rise in the O_2 uptake. In the present study HR responded immediately and 50 per cent of the HR change had on the average taken place during the first 10–15 s period. The concomitant small increase of $\dot{V}_{O_2}$ ... 1 l/min) was of a magnitude that would be found if the oxygen pulse (*i.e.* the product of stroke volume and a–v O_2 difference) had not changed during the same period. This is compatible with the findings of Raynaud *et al.* (1973), who observed only a slight increase in the a-v O_2 difference 10 s after the onset of supine exercise paired with a corresponding slight transient decrease in stroke volume. In the present study the major part of the on-response of $\dot{V}_{O_2}$ was observed after a delay of about 15 s. After that time $\dot{V}_{O_2}$ increased more rapidly than HR and cardiac output, indicating a widening of the arteriovenous O_2 difference due to a drop in the mixed venous O_2 content (Raynaud *et al.* 1973). Provided that the onset of work is associated with an immediate fall in P_{O_2} in the blood perfusing the working muscles, the above delay will reflect the time necessary for the blood to traverse the vascular segment between working muscles and the lungs.

The off-transient of $\dot{V}_{O_2}$ showed no change at all until about 15 s after the work-load change. Deoxygenated blood, returning during the first half circulation time after the end of exercise, may prevent a rapid fall of $\dot{V}_{O_2}$, and if at the same time, venous return is maintained, no fall at all of $\dot{V}_{O_2}$ would be observed as in the present case. The post-exercise increase in stroke volume, reported by Cumming (1972) to take place in supine subjects, may have contributed to maintain venous return despite the observed rapid fall in HR at the end of work, and so may probably the loadless pedalling movements by activating the muscle pump in the legs.

References

...LOW, J. H. J., R. GILBERT and G. H. BAULE, Effect of ventilation on oxygen transfer during early exercise. *J. appl. Physiol.* 1966. *21.* 810–818.

...OLIN, S. A. HOLMGREN and B. JONSSON, The effect of body position on the circulation at rest and during exercise, with special reference to the influence on the stroke volume. *Acta physiol. scand.* 1960. *49.* 279–298.

...ATTUNEN, H. and O. WIGERTZ, Dynamics of arterial oxygen tension in response to sinusoidal work load in man. *Acta physiol. scand.* 1971. *82.* 236–249.

...ROSS, SVETLANA and O. WIGERTZ, Transient dynamics of ventilation and heart rate with step changes in work load from different load levels. *Acta physiol. scand.* 1971. *81.* 54–74.

relative rather than the absolute work load. A comparison of the off-responses of HR between the two studies yields similar conclusions. The observation that off-responses of HR were slower than the corresponding on-responses is also in accordance with previous observations (Cerretelli, Sikand and Farhi 1966, Gilbert, Auchincloss and Baule 1967 Broman and Wigertz 1971) The over-all ventilatory responses were similar to those reported by Broman and Wigertz, and it was confirmed that the mean response of HR is considerably faster than that of V_E both in the on- and the off-transients.

Our finding that the increase in HR leads that of V_{O_2} is in accordance with the findings of Cerretelli *et al* (1966). This does not exclude, however that the peripheral oxygen uptake readjusts at the same rate as, or even slightly faster than cardiac output, as was found by Gilbert, Auchincloss and Baule (1967)

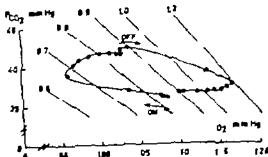
The mean response time (45 s) for the on response of V_{O_2} in the present study corresponds to a half time of 30 s, which is the value commonly found among previous investigators in the transition from rest to exercise (Henry and de Moor 1956, Margaria *et al.* 1965, Cerretelli *et al* 1966, di Prampero *et al* 1970 Whipp 1971 Whipp and Wasserman 1972).

The congruence between the on- and off-responses of $\dot{V}_{O_2}$ is by definition associated with an O_2 deficit O_2 debt ratio close to unity This is in agreement with Whipp, Seard and Wasserman (1970) who concluded that, using zero load as the base-line condition before and after steady-state submaximal work, the efficiency for anaerobic and aerobic work are equal The present results thus support the concept that the fast component of the O_2 debt consists of repayment of loans from stores of molecular O_2 and phosphagens (adenosine triphosphate and creatinephosphate), that were utilized at the onset of exercise.

There was a striking similarity between the time course of V_E and V_{CO_2} both with regard to short term and long term changes. The similarity in the short term fluctuations suggests a passive behavior of V_{CO_2} in a sense that rapid changes of CO_2 stores are induced by V_E fluctuations. The similarity in the long term changes suggests a tight functional coupling between the production of CO_2 and the response of V_E to submaximal work (*cf* Wasserman, Van Kessel and Burton 1967)

Responses of end-tidal P_{O_2}, P_{CO_2} and R

The discrepancies between the time courses of V_{CO_2} and $\dot{V}_{O_2}$ result in large changes of R following the onset and the termination of exercise (Fig. 2). These changes of R can be visualized together with the corresponding changes in the end-tidal gas tensions in an O_2-CO_2 diagram (Fig. 5) (Rahn and Fenn 1955). Using the terminology of these authors, the changes of end-tidal P_{O_2} versus P_{CO_2} can be described as a hypoventilation loop constituting the dominant response during exercise and recovery The end-tidal R values of the diagrams appear generally to be about 0.1 lower than those obtained from gas exchange measurements (Fig. 2), which discrepancy may reflect differences between end-tidal and mean alveolar gas due to the cyclic P_{O_2} and P_{CO_2} changes within each breath (Matell 1963, Rosenhamer 19[illegible]) P_{O_2} falls and P_{CO_2} rises towards the end of an expiration with an associated decrease of the instantaneous R value (Kim, Rahn and Farhi 1966) and may thus explain the observed differences between mean alveolar R and end tidal R



O_2–CO_2-diagram showing the changes ...tidal P_{CO_2} versus end-tidal P_{O_2} during ...t 5 min of exercise (on) and recovery ... Filled circles denote start values and ...circles indicate every 30 s. The super...d R-isopleths show that end-tidal R ... generally were some 0.1 R units ... than the corresponding R values com... from $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ (cf. Fig. 7).

...t changes ... time delays in the initial responses of HR and $\dot{V}_{O_2}$

...immediate rapid HR increase at the onset of exercise was originally described by Krogh ... Lindhard (1913) and it was thought to be associated with a rapid rise in the O... uptake. ...e present study HR responded immediately and 50 per cent of the HR change had on the ...age taken place during the first 10–15 s period. The concomitant small increase of $\dot{V}_{O_2}$ ...l/min) was of a magnitude that would be found if the oxygen pulse (i.e. the product of ...ke volume and a–v O_2 difference) had not changed during the same period. This is ...patible ...th the findings of Raynaud et al. (1973), who observed only a slight increase ...he a–v O_2 difference 10 s after the onset of supine exercise paired with a corresponding ...ht transient decrease in stroke volume. In the present study the major part of the on-...ponse of $\dot{V}_{O_2}$ was observed after a delay of about 15 s. After that time $\dot{V}_{O_2}$ increased more ...idly than HR and cardiac output, indicating a widening of the arteriovenous O_2 difference ...to a drop in the mixed venous O... content (Raynaud et al. 1973). Provided that the onset ...work is associated with an immediate fall in P_{O_2} in the blood perfusing the working ...scles, the above delay will reflect the time necessary for the blood to traverse the vascular ...ment between working muscles and the lungs.

The off-transient of $\dot{V}_{O_2}$ showed no change at all until about 15 s after the work-load ...ange. Deoxygenated blood, returning during the first half circulation time after the ...d of exercise, may prevent a rapid fall of $\dot{V}_{O_2}$, and if at the same time, venous return is ...intained, no fall at all of $\dot{V}_{O_2}$ would be observed as in the present case. The post-exercise ...crease in stroke ...olume, reported by Cumming (1972) to take place in supine subjects, ...ay have contributed to maintain venous return despite the observed rapid fall in HR at ...e end of work, and so may probably the loadless pedalling movements by activating the ...uscle pump in the legs.

References

...ONCLOS, J. H. J., R. GILBERT and O. H. BAULE, Effect of ...ntilation on oxygen transfer during ...ady exercise. J. appl. Physiol. 1966. 21. 810–818.

...ERHOLM, S., A. HOLMGREN and B. JONSSON, The effect of body position on the circulation at rest and during exercise, ...ith special reference to the influence on the stroke volume. Acta physiol. scand. 1960. 49. 279–298.

...LAMBRT, H. and O. WIGERTZ, Dynamics of arterial oxygen tension in response to sinusoidal work load ...n man. Acta physiol. scand. 1971. 82. 236–249.

...JARNAS, SVETLANA and O. WIGERTZ, Transient dynamics of ventilation and heart rate with step changes in work load from different load levels. Acta physiol. scand. 1971. 81. 54–74.

BROMAN, S., O WIGERTZ and M E. NYGÅRDS, A procedure for reduction of noise and artefacts in automatic processing of physiological measurement data. *Rep Lab. A lat Naval Med., Karol. Inst., Stockh.* 1970. 32 pp.

CARLSSON, A., G HANNAUER, T CAREY and P J HOLSBERG, *Handbook of Analog Computation*. Electronic Associates, Inc., Princeton, New Jersey 1965 389 pp.

CERRETELLI, P., R. SIKAND and L. E. FARHI, Readjustments in cardiac output and gas exchange during onset of exercise and recovery *J appl. Physiol.* 1966. *21* 1345–1350.

CUMMING, G R., Stroke volume during recovery from supine bicycle exercise. *J appl. Physiol.* 1972. 32 575–578.

GILBERT R., J H AUCHINCLOSS, Jr and G H BAULE, Metabolic and circulatory adjustments to unsteady-state exercise. *J appl. Physiol* 1967 *22*. 905–912.

HANSON, J S., B. S. TABAKIN and E. J CALDWELL, Response of lung volumes and ventilation to posture change and upright exercise. *J appl. Physiol* 1962. *17* 783–786.

HENRY F M and J C. DEMOOR, Lactic and alactic oxygen consumption in moderate exercise of graded intensity *J appl. Physiol* 1956. *8* 608–614

JONES, W B. R. N FINCHUM, R. O. RUSSELL, Jr and T J REEVES, Transient cardiac output response to multiple levels of supine exercise. *J appl. Physiol.* 1970. *28*. 183–189

KROGH A and J LINDHARD, The regulation of respiration and circulation during the initial stages of muscular work. *J Physiol. (Lond.)*. 1913. *51* 112 136.

KIM, T S., H RAHN and L. E. FARHI, Estimation of true venous and arterial P_{CO_2} by gas analysis of single breath. *J appl Physiol.* 1966. *21* 1338–1344

LINDBORG, B., O WIGERTZ and TH. ÖDMAN, A beat-to-beat heart-rate meter with linear analog output for muscular exercise studies. *Rep Lab. Aviat Naval Med., Karol. Inst Stockh.* Dec. 1969. 23 pp.

LINNARSSON, D Dynamics of pulmonary gas exchange and heart rate changes at start and end of exercise. *Acta physiol. scand.* 1974 Suppl. 415

LINNARSSON D and B. LINDBORG, Breath-by-breath measurement of respiratory gas exchange using on-line analog computation. *Scand. J clin. Lab. Invest* 1974. *34* 219–224

MARGARIA, R., H T EDWARDS and D B. DILL, The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *Amer J Physiol.* 1933. *106* 689–715.

MARGARIA, R., F MANGILI, F CUTTICA and P CERRETELLI, The kinetics of the oxygen consumption at the onset of muscular exercise in man. *Ergonomics* 1965. *8* 49–54

MARQUARDT D. W An algorithm for least squares estimation of nonlinear parameters. *J Soc. Ind. Appl. Math.* 1963. *11* 431–441

MATELL, G., Time-courses of changes in ventilation and arterial gas tensions in man induced by moderate exercise. *Acta physiol. scand.* 1963 *58* Suppl. 206.

DIPRAMPERO, P E., C. T M DAVIES, P CERRETELLI and R. MARGARIA, An analysis of O_2 debt contracted in submaximal exercise. *J appl. Physiol.* 1970 *29* 547 551

RAHN, H and W O FENN, *A Graphical Analysis of the Respiratory Gas Exchange*. Washington, D.C. Amer Physiol. Soc. 1955. 38 pp.

RAYNAUD, J H BERNAL, J P BOURDARIAS, P DAVID and J DURAND, Oxygen delivery and oxygen return to the lungs at onset of exercise in man. *J appl. Physiol* 1973 *35* 259–262.

ROSENHAMER, G Estimation of alveolar P_{O_2}. *Acta physiol. scand.* 1972. *84* 22 8.

SJÖSTRAND, T Determination of changes in the intrathoracic blood volume in man *Acta physiol. scand.* 1951 *22*. 114–128.

WASSERMAN, K., A. L. VAN KESSEL and G G BURTON Interaction of physiological mechanisms during exercise. *J appl. Physiol* 1967 *22*. 71–85

WHIPP B. J Rate constant for the kinetics of oxygen uptake during light exercise. *J appl. Physiol* 1971 *30*. 261 263.

WHIPP B. J C. SEARD and K. WASSERMAN, Oxygen deficit-oxygen debt relationships and efficiency of anaerobic work. *J appl Physiol* 1970. *28* 452–456.

WHIPP B. J and K. WASSERMAN, Efficiency of muscular work. *J appl. Physiol.* 1969 *26*. 644–648.

WHIPP B. J and K. WASSERMAN, Oxygen uptake kinetics for various intensities of constant-load work. *J appl. Physiol.* 1972. *33*. 351 354.

WIGERTZ, O A low-resistance flow meter for wide-range ventilatory measurement. *Respir Physiol.* 1969 *7* 263–270.

WIGERTZ, O. Dynamics of respiratory and circulatory adaptation to muscular exercise in man. *Acta physiol. scand.* 1971 *82*. Suppl. 363.

Acta physiol. scand. 1975. 95. 341–343
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...fluence of Substance P on the Response of Guinea Pig Ileum to Transmural Nerve Stimulation

By

P. Hedqvist and U. S. von Euler

...ance P (SP) seems to be closely associated with nervous tissue of various kinds. Thus, ...resence of SP in intestine has been correlated with the presence of intrinsic nerve plexuses ...intestinal wall (Pernow 1953) and large amounts of SP have been found in the dorsal ... of sensory nerves (Lembeck 1953), and in other nerves (Pernow 1953, Euler 1963). ... SP depolarizes motoneurons (Otsuka, Konishi and Takahashi 1972), sensory nerves ... and Lembeck 1973) and certain neurons within the central nervous system (Krnjevic ... Morris 1974, Phillis and Limacher 1974), it has been attributed a transmitter function ... role as modulator of nerve activity

...ecently oligodynamic concentrations of SP were found to increase the tone and to en... the stimulus response in the guinea pig vas deferens (Euler and Hedqvist 1974). In ... present study we wish to report on the influence of synthetic SP on responses of the ...itudinal muscle layer of the guinea pig ileum to transmural nerve stimulation and to ...ylcholine (ACh).

...uinea pigs were killed by blow on the head and the longitudinal muscle layer of the ...al ileum was carefully dissected free and placed in a 5 ml bath with Tyrode solution (for ...mposition, se Hedqvist and Euler 1972) aerated with 5% CO_2 in O_2. Field stimulation ... applied through platinum electrodes in the wall of the bath. The preparation, loaded ...h 0.25 g, was stimulated every minute with 1 2 pulses at 2 Hz through a Grass S4 Stimu...or in order to elicit a twitch-like contraction. The pulse duration was 1 ms and the volt... supramaximal. Contractions were recorded isotonically using a Harvard heart/smooth ...scle transducer coupled to Honeywell 190 inkwriter. Synthetic SP was kindly placed ...our disposal by Professor S. Leeman and used in aqueous solution of 1 or 10 µg/ml.

Single pulses were in most cases sufficient to elicit a brisk contraction. The stimulation ...rameters, as ell as the fact that the contraction response was abolished by atropine ... 10^{-7} M) and left unchanged by guanethidine (3 10^{-6} M), phentolamine (6 10^{-6} M) ...d propranolol (7 10^{-6} M) indicate activation of cholinergic nerves.

In concentration of 1.5 10^{-10} M SP regularly enhanced the contraction response to ...nsmural nerve stimulation and often increased the basal tone of the preparation. In...reasing the SP dose up to 7.5 10^{-10} M caused a dose-dependent increase in basal tone ...hereas the enhancement of induced contractions remained the same or increased mod-

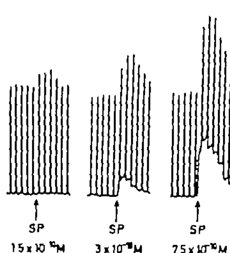


Fig. 1

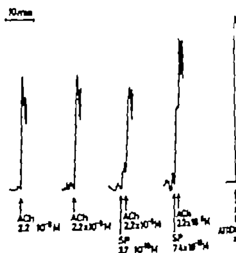


Fig. 2.

Fig. 1 Strip of longitudinal muscle layer from guinea pig ileum. Effect of SP on contraction resp transmural nerve stimulation (2 pulses, 2 Hz, 1 ms, supramaximal voltage) resumed each ...

Fig. 2. Strip of longitudinal muscle layer from guinea pig ileum. Effect of SP on contraction resp a standard dose of ACh, 2.2 10^{-8} M. To the right maximal contraction response (attenuated ... ACh.

erately (Fig. 1) Both effects were of relatively short duration inasmuch as they re maximum within 2–3 min and usually vanished after an additional 5 min. On the othe SP in the same concentrations as those found to enhance responses to transmura stimulation only occasionally and weakly enhanced contractions induced by ACh. In most cases the contraction response to ACh remained unchanged or even decre the presence of SP (Fig. 2).

Since the contractile effect of SP on intestinal smooth muscle is not affected by a or hexamethonium, an action directly on effector cells has been assumed (Pernow Similarly Lewis (1960) found the stimulant effect of SP on the gut to be much less by morphine than that of agents which act by stimulating nervous structures. On th hand, Beleslin and Varagic (1960) found that SP sometimes enhanced the contract the guinea pig ileum induced by ACh. Since hexamethonium inhibited this enhan they concluded the effect of SP to be partly due to sensitization of ganglionic cells

The present finding that SP much more readily enhanced contractions of the gu ileum resulting from nerve stimulation than from ACh administration suggests a p tional action of SP on cholinergic transmission, in addition to the direct stimulan on the smooth muscle cells. The high potency of SP together with its presence in wall invites the speculation of SP as a physiological modulator of smooth muscle in this tissue.

This study was supported by grants from the Swedish Medical Research Council, project no. 9 and from Stiftelsen Lars Hiertas Minne.

References

BELESLIN, D. and V VARAGIC, The effect of Substance P on the responses of the isolated guinea-p to acetylcholine, nicotine, histamine and 5-hydroxytryptamine. Arch. int. pharmacodyn. 1960. 1 327

..., U. S. Substance P in subcellular particles in peripheral nerves. *Ann. N.Y. Acad. Sci.* 1963, *104*, 449–461.

..., U. S. and P. Hedqvist. Effects of Substance P on the response of guinea pig vas deferens to ... stimulation. *Acta physiol. scand.* 1974, 90, 651–653.

...qvist, P. and U. S. v. Euler, Prostaglandin induced neurotransmission failure in the field-stimulated, isolated vas deferens. *Neuropharmacology* 1972, *11*, 177–187.

...n, H. and F. Lembeck, Effect of substances on paravascular pain receptors of the perfused rabbit ear ... Naunyn-Schmiedeberg's *Arch. Pharmacol.* 1973, *277*, R 34, Suppl.

...nević, K. and M. E. Morris, An excitatory action of Substance P on cuneate neurones. *Canad. J. Physiol. Pharmacol.* 1974, 52, 736–744.

...beck, F. Zur Frage der zentralen Übertragung afferenter Impulse. III. Mitteilung. Das Vorkommen und die Bedeutung der Substanz P in den dorsalen Wurzeln des Rückenmarks. *Naunyn-Schmiedeberg Arch. exp. Path. Pharmak.* 1953, *219*, 197–213.

...n, G. P. The inhibition by morphine of the action of smooth muscle stimulants on the guinea-pig intestine. *Brit. J. Pharmacol.* 1960, *15*, 425–431.

...uka, M., S. Konishi and T. Takahashi, A further study of the motoneuron-depolarizing peptide extracted from dorsal roots of bovine spinal nerves. *Proc. Jap. Acad.* 1972, *48*, 747–752.

...now, B., Studies on Substance P. Purification, occurrence and biological actions. *Acta physiol. scand.* 1953, 29, Suppl. 105.

...llis, J. W. and J. J. Limacher, Substance P excitation of cerebral cortical Betz cells. *Brain Res.* 1974, *69*, 158–163.

Acta physiol. scand. 1975. 95. 344–346

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Lactate Concentration in Type I and II Muscle Fibres during Muscular Contraction in Man

By

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Human skeletal muscles have two main fibre types, type I and type II Both have glycogen content, with glycolytic activity being more pronounced in type II fibre al the potential in type I may be enough to maintain a high rate of glycolysis. A t recruitment pattern of the different fibretypes may exist at onset of work and vario of exercise (Grimby and Hannerz 1968 Gollnick, Piehl and Saltin 1974).

It has not been established whether an accumulation of lactate occurs in both fib found in human skeletal muscle or mainly in the type II fibres. To investigate this I a method to determine lactate in fragments of single muscle fibres or fibres of the sa is introduced. In this report the method will be described together with preliminar from experimental procedures known to result in a lactate production.

5 healthy subjects participated in the study 3 subjects performed maximal bicycle exercise. Bio taken from each subject at 2 different situations in separate experiments. As indicated in Fig. were obtained at rest and after 10, 20 and 40 s after start of exercise and at exhaustion (3–4 min). O performed work of maximal intensity with a pressure cuff placed round one leg (300 mmHg samples were obtained from both legs at exhaustion (80 s) Two subjects performed isometric c at 50 % of MVC. Biopsies were taken in separate experiments after 20 s and at exhaustion (1 mi 1 and 5 is the same person.

The muscle biopsies were taken f om vastus lateralis (Bergström 1962) and frozen in liqu within 2–4 s.

An enzymatic fluorimetric method was used for analysing lactate. The reagent solution con mM glycine buffer 100 mM hydrazine-hydroxide, 1 mM NAD and lactic dehydrogenase 10 μ reaction was started by adding an aliquot of the sample. Concomitant blanks and standards w used The reaction time was 15–20 min.

Twentyfive biopsy samples containing lactate (1.5– 4.5 mmol/kg wet weight) were divided an tion was extracted in 3 M HClO as in earlier studies while the other portion was freeze-dried and in 1 M HCl.

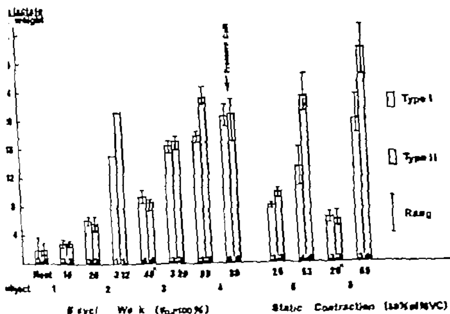
No significant difference was seen in lactat concentration between the two extraction (p < 0.05). When HCl-extraction was performed at 100°C f 2 h no change in lactate concen seen (p < 0.05). This would enable the determination of both lactate and glycogen from the sa (Essén and Henriksson 1974).

Analysis of lactate in pooled fibres

Muscle samples were freeze-dried and single f bres dissected out nd identified as type I or ATPase staining as described earlier (Essén *et al* 1975). 25–50 f bres of the same type were poole weighed (25–75 μg) and placed in 15 μl of 1 M HCl for 20 min. 8 μl of the supernatant as then 1 ml reagent solution and analysed for lactate as bo e.

Analysis of lactate in individual fibres

150 μl of reagent solution were used in this assay After the first reading on the fluorometer 1 μl of was pipetted on the inner wall of the tube bove the reagent solution and a single fibre (1 3 μg)



1. Lactate concentration is shown in type I and type II fibres in human skeletal muscle during rest at different times during bicycle work at maximal level and static contraction at 50% of maximal voluntary contraction (MVC). 6 subjects participated and 2 different samples were taken from each subject. The number within each bar indicates the number of samples of pooled fibres that were analysed. Note values are expressed in wet weight assuming a water content of 77% in the muscle.

the drop of HCl. The reaction was then started by mixing the drop of HCl with the reagent solution after ... 25 fibres were divided and lactate determined on each half. The coefficient of variation of the value was 18%.

The lactate concentration at rest and work on pooled fibre types with the method used in this study agrees well with results from the literature (Saltin *et al.* 1971; Diamant, Karlsson and Saltin 1968).

At rest the lactate concentration was 1.8 mmol/kg wet weight in both fibre types (Fig. 1). After 10 s of intense dynamic work the concentration had increased to 2.8 mmol/kg wet weight in both fibre types. A further rise in lactate concentration occurred during the exercise and reached 20 mmol/kg wet weight at exhaustion (3–4 min).

One of the subjects had a higher concentration in the type II fibres (only one pool from each fibre type). In the leg with blood flow occluded there was equal high lactate concentrations in both fibre types during maximal work (20 mmol/kg wet weight). The non-occluded leg had a 5 mmol/kg wet weight higher lactate concentration in type II fibres (the ranges for the samples does not overlap). The high lactate concentration in the leg with intact flow may be due to asymmetric division of work output between the two legs.

After 20 s of isometric contraction lactate concentration in both fibre types was similar but at exhaustion type II fibres had 10 mmol/kg wet weight higher lactate concentration than type I fibres in both subjects (the range for the samples does not overlap). The fact that type II fibres have a greater capacity for glycolysis may be related to this finding.

SUBJECT NO	EXPERIMENT	TYPE I		TYPE II	
		SINGLE FIBRES	POOLED FIBRES	SINGLE FIBRES	POOLED FIBRES
4	Dynamic work Exhaustion	15 8 2 1 (13 5 18 4) n_1 5	17 6 (16 7 18 2) 2 3	25 9 6.5 (16.2 34.0) n_1 11	22.9 (22.1–24 4) n_2 3
4	Dynamic work Exhaustion with pressure cuff	25 4 ± 5 1 (15 0–32 9) n_1 10	20.2 (19 0–21 9) n_2 6	26.7 4 5 (21 0–35) n_1 21	19 6 (17.0–22 8) n_2 6
5	Static contraction (50 MVC) 20	8 3 3 8 (4 0–15 5) n_1 16	7 9 (7 6–8 2) 2 3	12 1 2 8 (6 2 15 7) n_1 20	9.7 (9.0–10.8) n_2 3
5	Static contraction (50 MVC) 53 Exhaustion	18 8 ± 4 9 (7 2 23 6) n_1 13	13 2 (10 6–18 8) 2 4	27.2 3.6 (19 5–30 5) n_1 11	23.5 (20 9–26.0) n_2 4
6	Static contraction (50 MVC) 70	9 2 5 (3 1 13 0) n_1 18	6 2 (5 4–6 9) 2 3	7 0 2 8 (3 6–11 5) n_1 16	5.8 (4.5–7 1) n_2 3

TABLE I Mean value SD and range of lactate concentrations in single fibres (n_1 number of fibres, range in parentheses) and mean value for pooled fibres (n_2 number of samples of pooled f… Dynamic work 100 % V_{O_2} and static contraction 50 of maximal voluntary contractio…

Lactate was also determined on individual fibres in five biopsies and the mean value… well with those found on pooled samples, however a very marked variation outs… analytical error was observed within each fibre type (Table I).

As pointed out in the introduction a selective recruitment of muscle fibres (fibre… may occur in exercise. The large variation found for the lactate concentration of the ind… muscle fibres agree well with only certain muscle fibres being activated. A possibility… that at least part of the lactate found in some fibres may have diffused from an a… lactate producing muscle fibre.

This study was supported by grant from the Swedish Medical Research Council (04X-4554).

References

BERGSTRÖM, J Muscle electrolytes in man. *Scand. J clin Lab Invest* 1962. Suppl. 68.

DIAMANT B., J KARLSSON and B. SALTIN, Muscle tissue lactate after maximal exercise in man. *Acta scand.* 1968. 72. 383–384

ESSÉN, B. and J. HENRIKSSON, Glycogen content of individual muscle fibres in man. *Acta physiol…* 1974. 90. 645–647

ESSÉN, B., E. JANSSON, J HENRIKSSON, A. W TAYLOR and B. SALTIN, Metabolic characteristics… types in human skeletal muscle. *Acta physiol. scand.* 1975. 95 153–165

GOLLNICK, P D., K. PIEHL and B. SALTIN, Selective glycogen depletion pattern in human muscl… after exercise of varying intensity and at varying pedalling rates. *J Physiol. (Lond.)* 1974. 241 …

GRIMBY L. and J HANNERZ, Recruitment order of motor units on voluntary contraction, changes… by proprioceptive afferent activity *J Neurol. Neurosurg Psychiat* 1968. 31 565–573.

SALTIN, B., P D. GOLLNICK, B. O. ERIKSSON and K. PIEHL, Metabolic and circulatory adjustments… of maximal work. In. *Onset of Exercise* eds. A. Gilbert and P Guille, Univ of Toulouse, 1971

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The Effect of Stretch on Potassium Contracture Tension in Twitch and Slow Muscle Fibres of *Xenopus laevis*

By

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polarization of the surface membrane of a skeletal muscle fibre initiates a series of events ich ultimately leads to contraction of the fibre. The relation between membrane potential l force generation has been investigated by Hodgkin and Horowicz (1960) in isolated frog tch fibres by rapidly changing the external *potassium concentration*, $[K]_o$, and recording tension production associated with the ensuing step in membrane potential. The log $[K]_o$-sion relation is described by a steep S-shaped curve, starting at about −50 mV (20 mM K) l reaching a maximum at about −20 mV (80 mM-K). It has recently been reported onzales-Serratos, Valle and Cillero 1973) that extending a frog twitch muscle fibre from 2 to ∼2.6–2.8 µm sarcomere length increases the tension response to depolarizations by threshold K-concentrations (30–60 mM) by about 25%. The present report shows that a milar effect is obtained in *Xenopus* twitch fibres but not in slow muscle fibres.

ngle fibres were dissected from the doubtful [illegible] muscle of medium-sized clawed toads (*Xenopus laevis*) under darkfield illumination. The amount of light scattering particles was used as guide for selecting fibres of different functional types (Lännergren and Smith 1966). For the *twitch fibre* experiments, granular fibres located outside the tonus bundle were selected. For the *slow fibre* experiments, transparent fibres from the centre of the tonus bundle were used. Fibres selected to be of the twitch type were tested for their ability to give twitches and tetanus on electrical stimulation. The response of slow fibres was tested at the beginning of each experiment, the criterion for correct identification being local contraction on electrical stimulation and large, maintained contracture in 20 mM-K solution.

The selected *fibre was mounted in* narrow chamber with one *tendon held fixed and the other connected* to a [illegible] force transducer (AE 801, Akers Electronics, Horten, Norway). The resonance frequency of the transducer (in air) was 2 kHz and its compliance 0.013 m N^{-1}. The fibre could be observed through high-power microscope while suspended in the chamber. A cover-slip was placed on top and Leitz UMK 30 objective was used. Measurements of sarcomere length were made with the aid of calibrated ocular scale. Various solutions are introduced into the chamber by means of stop-cock system. Ringer solution (NaCl 115 mM, KCl 2.5 mM, $CaCl_2$ 2.0 mM, phosphate buffer 3.0 mM, pH 7.2) was continuously flowing at slow *rate* through the chamber between tests. A contracture of the fibre was induced by quick change (1–2 s) from Ringer solution to solution of increased K-concentration. Contracture solutions were prepared so as to keep the [K] [Cl] product constant (Hodgkin and Horowicz 1959). The experiments were performed at room temperature (21–24°C).

Fig. 1A and B show the effect of stretch from 2.25 µm (A) to 3.05 µm (B) sarcomere length on the mechanical threshold of a *twitch fibre*. The K-concentration used was 17.5 mM which was sub-threshold at the shorter length but elicited a relatively large contracture (25% of

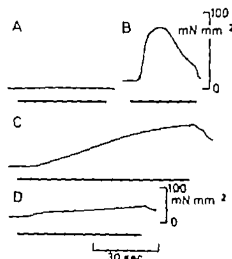


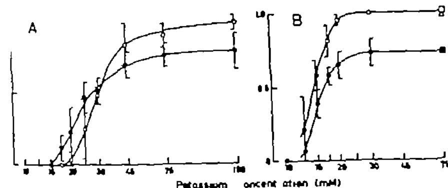
Fig. 1 Tension developed by a twitch fibre at 2.25 μm sarcomere length (*A*), and at 3.05 μm (*B*) in 17.5 mM-K solution applied during time indicated by horizontal bar. *C*, contracture tension of a slow fibre in 12.5 mM-K solution at ... μm sarcomere length, *D* In same solution at 3.10 μm

maximum tetanic tension) at the greater length. With larger positive polarizations contractures developed also at the shorter length but up to about 30 mM K larger tension responses were obtained after the fibre was stretched. Above 30 mM K the tension response was reduced by stretching the fibre. This was expected since it is well-known that the maximum tension is related to the amount of filament overlap (Gordon, Huxley and Julian ...) and overlap is decreased by stretching the fibre beyond 2.2 μm sarcomere length. The relation between $[K]_o$ and peak contracture tension for twitch fibres at two sarcomere lengths is presented in Fig. 2A. The results obtained on *Xenopus* twitch fibres agree with those obtained by Gonzales-Serratos et al. on frog twitch fibres. The displacement to the left of the $[K]_o$-tension curve after stretch was larger than that observed by them, but so was the stretch (35% of rest length as compared to 15–20% in their case). This suggests that even greater effects might be obtained by greater extensions.

Records C and D of Fig. 1 show the effect of stretch on the mechanical performance of a *slow* fibre at just suprathreshold positive polarization. In contrast to the twitch fibre the slow fibre response was larger at the shorter length. This was true also for all polarizations (Fig. 2B) The effect of stretch on the mechanical threshold was more difficult to determine since tension development was very slow at low K-concentrations but it was quite clear that a threshold-lowering effect like that seen in twitch fibres was absent in slow fibres.

Possible mechanisms behind the stretch effect in twitch fibres have been discussed by Endo (1972) who suggested that the Ca-sensitivity of the myofilaments is increased by stretch and by Gonzales-Serratos *et al.* (1973) who ascribed the effect to increased release of Ca from intracellular stores.

The present investigation reveals a clear-cut difference in response to stretch of twitch and slow *Xenopus* muscle fibres. Slow fibres differ from twitch fibres in several respects: (1) the mechanical threshold is lower and the $[K]_o$-tension relation steeper (Lännergren ...), (2) the dependence of tension on $[Ca]_o$ is stronger (Lüttgau 1963), and (3) the sensitivity towards replacement of extracellular Ca by other divalent ions is greater (Edwards and ...vic 1967) These differences most likely reflect differences in excitation-Ca release co...



...ᴛᴇ 2. Relation between peak contracture tension and [K$_o$] at 2 different sarcomere lengths, 2.25 µm (... circles) and 3.05 µm (filled circles). The symbols represent mean values from measurements on four ... fibres (A) and four slow fibres (B) (except at 75 mM-K where single measurements are given). ... bars indicate ± S.D. Ordinate: also 1.0 = tetanic tension (75–100 Hz) at 2.25 µm sarcomere length and 30 mM-K contracture tension (2.25 µm) in B.

...tempting to assume that the difference in the response to stretch is also due to a differ... in the activation mechanism but it cannot be excluded that the properties of the contrac... system of the two fibre types are different.

...nvestigation was supported by the Swedish Medical Research Council (Project No. 14X 3642) and ...ants from Karolinska Institutets Fonder.

References

...WARDS, C. and H. LORKOVIC, The roles of calcium in excitation-contraction coupling in various muscles of the frog, mouse, and barnacle. *Amer. Zoologist* 1967 *7*: 615–622.
...DO, M., Stretch-induced increase in activation of skinned muscle fibres by calcium. *Nature New Biol.* 1972 *237*: 211–213.
...MALES-SERRATOS, H., R. VALLE and A. CALZADO, Effect of muscle stretching on tension development and mechanical threshold during contractures. *Nature New Biol.* 1973 *246*: 221–222.
...RDON, A. M., A. F. HUXLEY and F. J. JULIAN, The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol. (Lond.)* 1966 *184*: 170–192.
...ODGKIN, A. L. and P. HOROWICZ, The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol. (Lond.)* 1959 *148*: 127–160.
...ODGKIN, A. L. and P. HOROWICZ, Potassium contractures in single muscle fibres. *J. Physiol. (Lond.)* 1960. *153*: 386–403.
...NNERGREN, J., Contractures of single slow muscle fibres of *Xenopus laevis* elicited by potassium, acetylcholine or choline. *Acta physiol. scand.* 1967 *69*: 362–372.
...NNERGREN, J. and R. S. SMITH, Types of muscle fibres in toad skeletal muscle. *Acta physiol. scand.* 1966. *68*: 263–274.
...TTMAN, H. C., The effect of calcium on potassium contractures of single muscle fibres. *J. Physiol. (Lond.)* 1963. *168*: 679–697

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Behavioural Signs of Emotional Excitement from Fastigial Stimul in "Chronically" Decerebrate Cats

By

Björn Lisander and Jan Martner

Stimulation of the cerebellar fastigial nucleus can elicit sham-rage in thalamic cats, a which is abolished by acute precollicular decerebration (Zanchetti and Zoccolini These findings can be taken as evidence of excitatory connections from the cerebel hypothalamic neuron pools involved in aggressive behaviour It is, however known also mesencephalic or even pontile cats can display signs of rage or fear albeit not integrated as the behaviour patterns seen in hypothalamic animals (Bard and Macht and several days have to elapse after decerebration before these responses can be obs As is the case in hypothalamic cats, they can be elicited by sound or nociceptive st suggesting that influences causing sham-rage exert at least part of their action at low stem levels, possibly *via* fairly unspecific actions on reticular neurons. It was therefore sidered of interest to study whether fastigial stimulation is capable of eliciting emot coloured behavioural patterns also in "chronically" decerebrate cats.

Six cats were operated under combined ketamine and pentobarbital anesthesia, strict aseptic precautions. The right carotid artery and the right external jugular vein cannulated with silicone rubber catheters with their tips placed in the aortic arch an right atrium, respectively The other catheter ends were drawn out through the skin lying the back of the skull The latter was opened over the occipital lobes which we moved, permitting egress of cerebrospinal fluid through the lateral ventricles. The stem was then cut at the predetermined level by a blunt spatula and the wound was c In subsequent days the animals received electrolytes, glucose solution, amino acid lipid suspension i.v The arterial catheter was flushed daily with heparin solution. He crit and serum potassium and sodium were followed and if necessary corrected. Overhe was prevented by a fan, regulated by a rectal thermistor keeping body temperatu about 35°C, which was increased to 37–38° during the observations of behaviour Alt days the animals were lightly anesthetized with pentobarbital and the fastigial presso (for references see Martner 1975) was stereotaxically localized and stimulated dur cording of blood pressure. The electrode was cemented in place. After at least 24 hour electrode was again stimulated and the behaviour of the animals was observed. All an treated in this way were kept for 6–7 days after decerebration and their brains were fixed in formaldehyde for subsequent macroscopic and histological examination.

of the cats the brain stem was transected through the lower mesencephalon and in the 3 cats at the junction between the mesencephalon and the pons. Strong extensor rigidity ...ped within a few hours after decerebration and tonic neck and labyrinthine reflexes readily elicited. When undisturbed, the animals lay on their side. Some days after the ...tion they displayed righting of the head, often induced by handling. Some of the ani... could right their forelimbs as well as their head.

...four of the animals, emotionally coloured response patterns were observed and in two ... in this occurred "spontaneously" or was easily triggered by touch or vibration of the ... In the two other animals such beha-iours were elicited first when nociceptive stimuli ... applied. The responses consisted of attempts to right head and shoulders, protrusion ...ws, running movements with all legs, lashing of the tail and an increased respiratory ... In one cat there was vocalization as well. Increases in heart rate and blood pressure and ...ction of the nictitating membrane paralleled these somatomotor changes.

...e of the animals were implanted with an electrode in the fastigial pressor area. Stimula... (square waves, 50 Hz, 1 ms and 0.03–0.2 mA) caused marked increases in blood pressure ... heart rate and all the animals displayed the above described somatomotor changes, in ... or partly. Thus, all animals made running movements with their legs and increased ... respiratory rate and three of them righted themselves with head and shoulders and ...ently tried to escape. One low mesencephalic animal reacted ...ery vividly ... had to be ...ped falling off the table, growled and urinated. These reactions were entirely stimulus-...bound and as a rule ceased within 20 s after stimulation. As a rebound phenomenon, ...lowing and chewing movements were regularly observed.

...mulations causing marked pressor responses induced a somatomotor pattern, entirely ...he above described type and postural reactions (Hare, Magoun and Ranson 1937) were ... apparent, neither during nor after stimulation. If stimulation intensity was decreased, ...tural responses could be observed initially in some animals, only to be followed by the ...tionally coloured changes. In deteriorated preparations, in which the emotional re...ses no longer could be evoked by nociceptive stimuli, postural changes were seen both ...ing and, as a rebound phenomenon, after the stimulus, especially at intense stimulation.

... 6 additional animals were decerebrated acutely under ether anesthesia. Fastigial pressor ... stimulation caused, as expected, increased blood pressure and heart rate. However the ...otionally coloured somatomotor changes observed in the chronically decerebrate cats ...ld not be induced, except for an increased respiratory rate.

In agreement with earlier findings, the present experiments have shown that even decere-...te animals can display fragments of flight or attack beha-iour though these responses ... not as ...ell integrated and easily elicited as in hypothalamic animals (Bard and Macht ...58). The somatomotor changes were paralleled by autonomic changes such as increases in ...art rate and blood pressure and retraction of the nictitating membranes.

Similar behavioural changes could be elicited by stimulation of the fastigial pressor ...ea. These findings suggest that although fastigial stimulation can induce aggressive ...haviour in thalamic animals, this cannot be taken as evidence of direct cerebellar projec-...ons to the diencephalon. The fact that acute decerebration can prevent these behavioural ...anges may be explained by the inflicted trauma (Monakow 1914, p. 26). It is unclear

whether the state in "chronically" decerebrate animals represents a recovery to non bility of brain stem structures or whether supersensitivity develops. Whichever the present study has demonstrated cerebellar projections to bulbar and mes neuron pools involved in emotional behaviour possibly the same which can be a intact animals or hypothalamic preparations.

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References

BARD P and M B. MACHT The behaviour of chronically decerebrate cats. In. *Ciba Foundation on the neurological basis of behaviour* Ed. G E. W Wolstenholme and C. M. O'Connor. J. & A Ltd., London, 1958, 55–75.

HARE, W K. H. W MAGOUN and S. W RANSON, Localization within the cerebellum of reactio cerebellar stimulation. *J comp. Neurol.* 1937 *67* 145–182.

MARTNER, J Cerebellar influences on autonomic mechanisms. An experimental study in t special reference to the fastigial nucleus. *Acta physiol. scand.* 1975 Suppl. 425.

MONAKOW C. von, *Die Lokalisation im Grosshirn und der Aufbau der Funktion durch kort* J F Bergmann, Wiesbaden, 1914, 1 1033.

ZANCHETTI, A. and A. ZOCCOLINI, Autonomic hypothalamic outbursts elicited by cerebellar *J Neurophysiol.* 1954, *17* 475–483.

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Reversibility of Effects of Very Hypotonic Fluids on *In Vitro* Frog Gastric Mucosa. A Functional and Morphological Study

By

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Abstract

HELANDER, H. F., S. S. SANDERS, L. L. SHANBOUR and W. S. REHM. *Reversibility of effects of very hypotonic fluids on in vitro frog gastric mucosa: A functional and morphological study.* Acta physiol. scand. 1975. 95. 353–363.

Functional and morphological properties of the *in vitro* frog gastric mucosa were studied during and after exposure to very hypotonic (25 mOsM) solutions. Within 20 min the acid secretory rate decreased to zero, but it returned to normal levels after isotonic fluids had been restored. The potential difference (PD) dropped within the first minutes after the exposure to hypotonic solutions, and became inverted. Following the return of isotonic conditions the PD increased to levels higher than in the controls. The electrical resistance increased about 10-fold during the hypotonic period, but decreased to near normal values when isotonic conditions were restored. By light and electron microscopy the cells of the hypotonic mucosae appeared greatly swollen, and the alterations were assessed by morphometric methods. The gland lumina were almost obliterated, and the lamina propria was reduced to about 60 % of its former volume. After the return to isotonic conditions normal morphology was restored. It is conceivable that the great increase in resistance during the hypotonic period was caused by the occlusion of the gland lumina. Quantitative analyses of the Na, K, and Cl tissue concentrations indicated a large loss of these ions during the hypotonic state. Presumably the epithelial cells in the hypotonic mucosae avoid bursting by rapidly letting large numbers of ions out, which results in a cellular osmolarity close to that of the bathing fluids.

Key words: Gastric mucosa, frogs, hypotonic solution, electrophysiology, microscopy

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In a previously unpublished work (Sanders and Rehm) we found that by bathing the *in vitro* frog gastric mucosa with half-isotonic fluids the functions of the gastric mucosa were not markedly impaired. This raised the question as to whether the frog gastric mucosa could continue to function in very hypotonic bathing fluids. We determined the effect of hypotonic fluids which contained the same concentrations of K, Ca++, Mg++ and H as the control solutions but had a total osmotic pressure of from 25 to 30 mOsM (isotonic ≈ 200) and found that H secretion was abolished. We expected that the mucosal cells would be cytolyzed and no recovery of function would be possible, but instead, after restoration of the control isotonic fluids, the H rate recovered. We then studied the effects of hypotonic media on the electrical parameters, the ionic content and the histology of the gastric mucosa. The

whether the state in "chronically" decerebrate animals represents a recovery to normal ex- bility of brain stem structures or whether supersensitivity develops. Whichever the ca the present study has demonstrated cerebellar projections to bulbar and neuron pools involved in emotional behaviour possibly the same which can be activated intact animals or hypothalamic preparations.

This research has been sponsored by grants from the Swedish Medical Research Council (No 14C and by grants from the Medical Faculty of Göteborg.

References

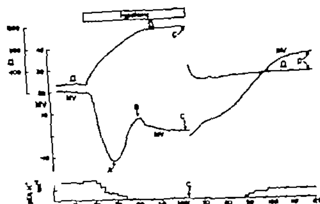
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HARE, W K., H. W MAGOUN and S W RANSON, Localization within the cerebellum of reactions to lo cerebellar stimulation. *J comp. Neurol.* 1937 67 145–182.

MARTNER, J., Cerebellar influences on autonomic mechanisms. An experimental study in the cat special reference to the fastigial nucleus. *Acta physiol. scand.* 1975. Suppl. 425.

MONAKOW C. von, *Die Lokalisation im Grosshirn und der Aufbau der Funktion durch kortikale He* J F Bergmann, Wiesbaden, 1914. 1 1033.

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...1 Tissue resistance in ohms 1.3 cm² of mucosa, potential ...ference in mV (nutrient side positive), and ...d secretory rate in one of the ...periments. For further explana... ...n see text.

...h at 4°C, rinsed again in nutrient, dehydrated in rising concentrations of ethyl alcohol, and finally em...bedded in Epon or Araldite.

Light microscopy was performed on one micron thick sections stained with toluidine blue. For the ...ometric analyses the point-counting method was used (Weibel *et al.*, 1969) with ... square lattice mounted ... the eyepiece of the microscope. 5 sections from each of 5 animals were quantitated for each of the 3 ...ates, "control" "hypotonic" and "return". For each animal the 5 sections were taken from different ...ts of the gastric mucosa. The relative volumes estimated in this study were: epithelial cell volume, inter...stitial tissue (... lamina propria) and, except for the hypotonic condition, the lumen.

Electron microscopy was performed on thin (... 80 nm) ultramicrotome sections, ...hich were contrasted ...ith lead hydroxide and uranyl acetate.

Results

Functional results

...n preliminary experiments we found that if the hypotonic state (hypotonic solution on both ...ides) ...as continued for periods of over an hour before returning to isotonic solutions, ...he H ... secretory rate either did not return or returned to only a small fraction of its original ...rate. However there was good return of function after exposure of less than 1 h, and thus ...a period of about 30 min was used as the standard time of exposure to the hypotonic fluid ...(actual range was from 28 to 46 min).

Fig. 1 shows a typical experiment in which isotonic fluids were replaced with hypotonic ones on both sides. The H ... secretory rate started decreasing within a few minutes and reached a zero rate in about 20 min. After the return of the isotonic fluids the H ... rate returned in all of the 9 expts. (see Table II) after a latent period of about 15 min.

With hypotonic fluids the resistance increased and continued to increase over a period of about 15 min and in some experiments reached extraordinarily high levels. Frequent measurements of the resistance (every 10 s) revealed no transient decreases in the resistance, ...hich could be expected if the cells had become damaged and leaky. Following the return to isotonic conditions the resistance decreased rapidly to a level near the original control level.

The PD during the hypotonic period showed an initial marked drop of about 30 mV (the nutrient side became negative by about 12 mV), and this was followed by a rebound

TABLE I Composition of incubation fluids (in mM).

	Secretory isotonic	Secretory hypotonic	Nutrient isotonic	Nutrient hypotonic
Solution No.	1	2	3	4
K^+	4	4	4	4
Na^+	100	7	102	7
Cl^-	104	6	101	6
HCO_3	0	0	5	5
SO_4	0	2.5	0.8	0.8
Ca^{++}	0	0	1.0	1.0
Mg^{++}	0	0	0.8	0.8
HPO	0	0	1.0	1.0
Glucose	0	0	5.0	5.0
Sucrose	0	6	0	0
Osmolarity (mOsM)	208	25.5	220.6	30.6

Calculated on assumption osmotic coefficients are unity actual osmolarities ≃ 3 to 8% less.

primary purpose of the present paper is to present the results of these findings. Prelim reports of this work have been given (Sanders and Rehm 1968, Sanders, Shanbou Rehm 1970).

Methods

The experiments were performed on *Rana pipiens* with an *in vitro* method described in detail el (Rehm 1962, Rehm *et al.* 1963). Two pairs of electrodes were used, one pair for sending current the mucosa and the other for measuring the PD. The resistance was determined as the ratio of the ch PD (after 0.5 s) per unit of applied current (Kidder and Rehm 1970, Noyes and Rehm 1970). The was applied first in one direction and then in the other direction there was no significant rectif The solutions used in these experiments are given in Table I. The resistance values were correc solution resistances on the two sides (36 ohm cm² for the isotonic solutions and 250 ohm cm² for th tonic solutions). After control period in isotonic bathing media (No. 1 and 3, Table I) we placed hy solutions (No. 2 and 4 of Table I) on both sides of the mucosa for periods of about 30 min and then c the fluid to the original controls (No. 1 and 3 of Table I). The original controls are referred to as "iso the experimental period as "hypotonic" and the return to isotonic conditions as returns. Histal 10^{-4} M was used in all nutrient solutions. The gas was 99% O_2 1% CO_2 so that we could use HCO_3 rather than 25 HCO_3 to maintain normal pH on the nutrient side. The use of 5 mM HCO_3 than 25 mM HCO_3 enabled us to achieve a tonicity around 30 mOsM. It should be pointed out going from 95% O_2–5% CO_2 with 25 mM HCO_3 to 99% O_2 1% CO_2 and 5 mM HCO_3 the H sec rate is reduced (Sanders, Hayne and Rehm 1973, Kidder 1974). However with 99% O_2 1% CO_2 an easily measured and relatively stable H secretory rate. The Na, K and Cl concentrations of the and the hypotonic mucosas were determined by flame photometry and the Cotlove chloridimetry tec by a method previously described (Shanbour, Davis and Rehm 1970). In preparing the tissue exce and loose mucus were removed by gently wiping the tissue with a glass rod. We did not remove the mucous coat because we previously found that attempts to do so result in loss of a substantial num surface cells (Canosa and Rehm 1968).

Histological methods

Fixation for morphological analyses was accomplished by adding a mixture of glutaraldehyde and (Peracchia and Mittler 1972) to the secretory and nutrient solutions simultaneously; the final concent of these compounds were 1 and 0.1% respectively. After fixing for 3 h, the chamber the m were removed, rinsed in nutrient solution for 1 h, postfixed in an ice-cold 1% OsO_4 solution in a tr

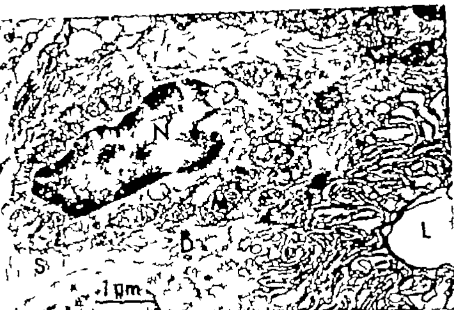


Fig. 2. Oxyntic cell from frog gastric mucosa incubated in isotonic solutions. An extensive network of microvilli and leaflets extends from the cell into the lumen (L) of the gastric tubule. Interdigitations are seen between the lateral cell membranes. The intercellular space (S) is up to about 0.3 µm in this figure. D, desmosome, M, mitochondrion, N, nucleus. × 16 000.

than in the isotonic oxyntic cells, and portions of the apical cytoplasm were frequently quite electron lucid. The intercellular spaces between neighboring oxyntic cells had shrunken to roughly 20 nm—the same as between the surface epithelial cells. The shrinkage of the lamina propria appeared uniform. The distance in the sections between neighboring glands varied considerably but interglandular regions of ~25 µm were frequently seen extending from the base of the glands to the surface epithelial cells.

The ultrastructure of the epithelial cells after *return* to isotonic solutions (Fig. 4) was essentially the same as in control isotonic mucosae. The surface epithelial cells exhibited a more or less flat apical surface with a few short microvilli. Numerous dense secretory granules were packed beneath this membrane, and the empty-looking regions of cytoplasm had disappeared. Intercellular spaces were still roughly 20 nm between the surface epithelial cells, but had returned to about 200 nm between the oxyntic cells (as in the isotonic mucosae). The gland lumina appeared somewhat wider than in the isotonic mucosae.

By light microscopy the isotonic (Fig. 5) and the return mucosae were similar but the hypotonic mucosae (Fig. 6) exhibited swollen cells. This was most easily seen on the surface of the mucosae, where the epithelial cells were frequently seen to bulge into the gastric cavity. At deeper levels of the hypotonic mucosae most gland lumina were evidently obliterated by swollen oxyntic cells. In some cases the regions where gland lumina could be expected were occupied by almost translucent material, which made it very difficult to determine whether the lumen was obliterated by a cell or not. Consequently it was not possible to

TABLE II Summary of physiological data from 9 return expts. Letters A though D refer to the intervals indicated on Fig. 1 A is for the nadir of the PD B the peak of the rebound of th C the time immediately before return to the isotonic condition, and D the time after a ste had been reached in the isotonic fluids.

	PD (mV)					Resistance (Ω cm²)			H Rate (μEq h⁻¹	
	Origin	At A	At B	At C	At D	Origin	At C	At D	Origin	At C
Average	24.4	−6.9	10.1	4.1	33.3	131	1 211	263	2.13	0
±S.D	7.2	5 9	6.7	6.3	11.5	21	207.8	223	0.37	
Range	13 to 36	−16 to 1	to 19	−3 to 14	14 to 46	104 to 160	871 to 1 547	117 to 850	1.68 to 2.64	

and then a small decline. Following the return to the isotonic conditions the PD m showed a negative spike which was then followed by a marked increase in the PD, a the end of the experiment it was about 15 mV greater than its values in the control condi

As shown previously (Spangler and Rehm 1968) the time constants for diffusion of molecules via the diffusion barriers separating the bathing fluids from the mucosa layer is about a minute or less. Thus the mucosal cell layer is exposed to the full imp the hypotonic fluids within a matter of a few minutes.

Table II presents a summary of 9 expts. In every experiment the H^+ secretory rate dec to zero during the hypotonic state and was reestablished following the return to iso conditions. The resistance showed an approximately 10-fold increase in the hypo state and recovered to a level which was slightly higher than the original level. The PD (at D) in 6 out of 9 expts. was about 20 mV higher than the original level in 3 it was lower

Morphological results

The ultrastructure of the normal frog gastric mucosa has previously been describ several authors (Geuze 1971 Sedar 1965 Forte, G M Limlomwongse and Forte, 1969), and our findings on the *isotonic* mucosae did not differ significantly from earlier results. Since much recent interest is devoted to transintercellular pathways, it sh be noted that the intercellular space between neighboring surface epithelial cells was gen in the range of 20 nm. However between adjacent oxyntic cells, the intercellular spac much wider up to a few hundred nm (Fig. 2) This space was very irregularly formed, limited apically by the terminal bar region. Desmosomes were frequently found bet surface epithelial cells, but were not so common between oxyntic cells.

The *hypotonic* mucosae exhibited a marked swelling of the cells the apical surface o surface epithelial cells was convex, being semispherical with an absence of microvil some instances the plasma membrane was broken In the unbroken cells the secretory gra were mixed with regions of very low electron density and flocculent or weblike mat The oxyntic cells were also swollen, and like the surface epithelial cells abnormalities more in the apical portions of the cells (Fig. 3) In most cases the gland lumen was pletely obliterated The ground cytoplasm and nucleus were generally lower de



Fig. 4. Survey of gastric mucosa from return stomach. This mucosa looks essentially similar to the "normal" mucosa, but the lower gland lumina appear somewhat dilated. L, lumen of gastric gland; MC, mucous neck cell, P gastric pit. 300.

hypotonic mucosae was 14.1 (S.D ±0.8) while for the isotonic mucosae it was 11.3 (S.D ± 0.8). Assuming no change of dry weight in the hypotonic mucosae then on the basis of the above figures it follows that the volume for the hypotonic mucosae was 1.25 the volume of the isotonic controls. From a comparison of the actual volumes with the assumptions that the increase in volume was uniform (*i.e.* the same for the epithelial cell portion as for the rest of the mucosa) it follows from our morphometric data that the epithelial cell volume of the hypotonic mucosae (0.75 1.25 0.94) was about twice that of the controls (0.48 1.0 0.48), and the interstitial fluid volume was about 60% of that of the controls.

Table IV also shows the K, Na and Cl content of the mucosae. Three other hypotonic experiments were performed in which the Na and Cl concentrations were slightly different in the hypotonic secretory solution than in the nutrient solution. The results of these latter experiments were essentially the same as those in Table IV.

Other orkers report lower values for Na and higher values for K in the in vitro frog gastric mucosae (*e.g.* Davenport 1963, Davenport and Alzamora 1962). However these workers removed the adherent mucous coat and since we did not remove this coat our values would be expected to be different.

Discussion

Many more questions have been raised by the results of the foregoing experiments than could possibly be answered within the scope of a single paper. It is clear that the frog gastric mucosa can recover after exposure of the mucosa to very hypotonic fluids for periods of



Fig. 3. Cross section of gland tubule from "hypotonic" mucosa. At this level of the mucosa the gland is made up of oxyntic cells. These cells are swollen to the extent that the lumen is totally obliterated, its former place is indicated in the center of the tubule (L). Mitochondria (M) and cell nuclei (N) are seen but the ground cytoplasm appears vacuolated or dissolved. The intercellular spaces are much more narrow than in the isotonic mucosae. In comparison with these also the nucleus seems to be a lost some of its electron density. × 3 000.

obtain meaningful morphometric values for the relative volumes of the lumen in the hypotonic mucosa. For the subsequent calculations of water content (see p. 12) it was of interest to estimate the volume of the epithelial cell layer and lamina propria in relation to the total volume of the chambered tissue flap. In the controls, the epithelial cell layer plus lamina propria occupied roughly 56% (S.D. 15.9) of the total tissue volume, hence the muscularis mucosae plus the portion of the submucosa included in the preparation occupied 44%. In the hypotonic and in the returns the corresponding values for the epithelial cell layer plus lamina propria were 51% (S.D. 8.4) and 52% (S.D. 4.1), respectively.

The relative epithelial cell volume + the volume of the gland and pit lumina (see Table II) were significantly larger in the hypotonic mucosae than in the isotonic ($p < 0.005$). Moreover, the relative epithelial cell volume was significantly larger in the returns than in the isotonic ($p < 0.01$). Also the relative lumen volume was significantly larger in the returns than in the isotonic ($p < 0.05$).

Water and electrolyte content of isotonic and hypotonic mucosae

The water content of the hypotonic mucosae was significantly greater than that of the isotonic controls (see Table IV). Thus, the average ww/dw (wet weight / dry weight) for the

TABLE III. Morphometric data on frog gastric mucosa. Values are given in per cent of total volume of epithelial cells and lamina propria (means ± S.D., n = 5).

	Isotonic	Hypotonic	Return
Epithelial cells	48.4 ± 9.1	—	63.0 ± 3.2
Gland and pit lumen	3.5 ± 0.7	—	11.4 ± 6.0
Epithelial cells + lumen	52.0 ± 9.5	75.4 ± 2.8	74.4 ± 5.5
Lamina propria	48.0 ± 9.5	24.6 ± 2.8	25.6 ± 5.5

calculated value of 80 down to about 25 mOsM. Although we are not able to arrive at precise values for the loss of ions from the cells the data in Table IV are compatible with the idea that there is sufficient loss of electrolyte from the cells to bring their osmolarity down to the level of the hypotonic fluid. Since it is generally assumed that mammalian cells would burst if a sizable hydrostatic pressure develops, it seems reasonable to believe that a substantial hydrostatic pressure never develops between the cells and the ambient media. Our tentative explanation is that the increase in water content of the cells and the loss of electrolytes result in a cellular osmolarity equal to that of the hypotonic media.

Analysis of the resistance changes during the hypotonic state

The resistance increases seen in the hypotonic experiments are in general larger than we have seen with other experimental conditions. In this section we present a possible explanation for the very large increases in resistance which is based on our findings and upon other findings presented in preliminary form (Sanders and Rehm 1968, Sanders, Shanbour and Rehm 1970). This explanation is no more than a tentative working hypothesis. First the question arises as to whether the very large increase in resistance is due to the high resistance of the hypotonic fluids. Using either the secretory or the nutrient hypotonic fluids in the chamber without a mucosa we found a resistance of about 250 ohm cm² which is about 210 ohm cm² greater than the resistance with isotonic solutions in the chamber in the absence of a mucosa. Calculations of the resistance of the gastric mucosa if it was replaced with a hypotonic solution reveal that the resistance would be about 50 ohm cm². Thus, whether we subtract 210 ohm cm² or 160 ohm cm² (210–50) makes little difference from the point of view of explaining the very large increase in resistance with the hypotonic media. Con-

TABLE IV. Water, K, Na, and Cl⁻ contents in isotonic and hypotonic mucosae (Means ± S.D., n = 4). WW = wet weight, DW = dry weight.

Conditions	WW/DW	K mEq/kg H_2O	K mEq/kg DW	Na mEq/kg H_2O	Na mEq/kg DW	Cl^- mEq/kg H_2O	Cl mEq/kg DW
Isotonic	11.3 ± 0.6	32.6 ± 5.8	336 ± 66	116.8 ± 4.0	1 202 ± 100	92.6 ± 3.0	953 ± 84
Hypoton.	14.1 ± 0.8	18.4 ± 3.4	241 ± 30	14.4 ± 1.8	189 ± 28	9.1 ± 1.6	120 ± 16



Fig. 5



Fig. 6

Fig. 5 Light micrograph of frog gastric mucosa incubated in isotonic solutions. The lumina (L) of the tubules are clearly visible in the basal half of the mucosa. They open into the gastric pits (P). Surfa thelial cells with dark secretory granules border the pits as well as the surface of the mucosa. LP l propria MM muscularis mucosae. 40

Fig. 6. Light micrograph of hypoto ic mucosa. The cells of the gastric glands are greatly swolle much less stained than n the isotonic mucosae. Clea cations are seen in the gland tubules, bu often difficult to decide whether these are gland lumina (L) or swollen projections from the oxyntic Due to the swelli g the lamina propria (LP) appears greatly reduced. The surface epithelial cells are sc bulge into the gastric ca ity Also the cells of the muscularis mucosae (MM) are swollen. 40.

30 min. The morphological findings indicate that the gastric cells swell in hypotonic me and that the interstitial fluid decreases. When the increase in the total volume of the muc in the hypotonic solutions is taken into account the cells swell about 2 times (or mc (as shown in the results p. 10) The increase in volume would undoubtedly be due to additi of water to the cells and assessing a dry weight to cells of 30% the water content of the ce would increase by about 2.5 times. So if no solutes exited from the cells the expected osm larity of the cells (with a 2.5 increase in water content) would be about 80 mOsM. Th osmolarity of the hypotonic media is about 25 mOsM so for the cells to have the sam osmolarity as the hypotonic media there must be sufficient loss of lutes to bring th

...m, W. S., T. L. Davis, C. Chandler, E. Goodman, J. and A. Bannerman, Frog gastric mucosae bathed in Cl^--free solutions. *Amer. J. Physiol.* 1963, 204, 233–42.

...nson, S. S. and W. S. Rehm, Effect of hypotonic solutions on in vitro frog gastric mucosa. *Biophys. J.* 1968, 8, WPR.

...nson, S. S., V. B. Hayne, Jr. and W. S. Rehm, Normal H rates in frog stomach in absence of exogenous CO_2 and note on the pH stat method. *Amer. J. Physiol.* 1973, 225, 1311–1315.

...nson, S. S., L. L. Shanbour and W. S. Rehm, Resistance changes of in vitro frog gastric mucosa bathed in very hypotonic fluids. *Biophys. Soc. Abstr.* 1970, 10, 32 a.

...ar, A. W. Fine structure of the stimulated oxyntic cells. *Fed. Proc.* 1965, 24, 1360–1367

...anbour, L. L., T. L. Davis and W. S. Rehm, Electrolyte content of the corneal stroma of the bullfrog. *Proc. Soc. exp. Biol. (N.Y.)* 1970, 133, 11–13.

...nvalle, S. G. and W. S. Rehm, Potential responses of nutrient membrane of frog stomach to step changes in external K and Cl concentrations. *Biophys. J.* 1968, 8, 1211–1227

...ibel, E. R., W. S. Stäubli, H. R. Gnägi and F. A. Hess, Correlated morphometric and biochemical studies on the liver cell. I. Morphometric model, stereological methods, and normal morphometric data for rat liver. *J. cell Biol.* 1969, 42, 68–91

sequently if the mucosa was replaced with hypotonic fluids and there were no geomet considerations we would not expect such a large increase in resistance. Furthermore, if t were no changes in the geometry of the cells and the cytoplasmic resistance increased al 7 fold this should make little difference in the magnitude of the resistance because the re ance across epithelia is primarily a function of the resistance of the limiting membran the cells and not of the cytoplasm. Therefore, we have to look at changes in the geom Our tentative hypothesis is that the surface cells (at least under the hypotonic condit have a very high resistance and that the low resistance seen normally with isotonic f (100 to 200 ohms cm²) is due to a low resistance of the tubular cells. Our major postul that the very high resistances seen with hypotonic fluids is due to a partial or almost com occlusion of the lumen of the tubular cells. The big range of the resistance values fou Table II might then reflect the degree of occlusion of the lumina. Complete occlusion w result in an essentially infinite resistance via the lumen. In a previous electron microsc study the total surface area of the secretory membranes (including gastric pits, gl microvilli etc.) of the *in vitro* frog gastric mucosa was found to be about 210 time macroscopic area and that of the oxyntic cells alone 190 times (Helander *et al.* 1 Hence complete occlusion of the lumen would result in a reduction of roughly 90 % c surface area (190/210 100) available for the passage of current. Assuming that the secr membrane surfaces all have the same resistance in terms of ohms for a cm² of actual the resistance would be expected to increase by about 10-fold which is approximately we found experimentally

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References

CANOSA, C. and W. S. REHM, Microelectrode studies of dog's gastric mucosa. *Biophys. J.* 1968, *8*, 41

DAVENPORT H W Sodium space and acid secretion in frog gastric mucosa. *Amer J Physiol.* 196 213–216.

DAVENPORT H W and F ALZAMORA, Sodium, potassium, chloride, and water in frog gastric m *Amer J Physiol.* 1962, *202*, 711 715.

FORTE, G M L LIMLOMWONGSE and J G FORTE, The development of intracellular membranes co tant with the appearance of HCl secretion in oxyntic cells of the metamorphosing tadpole. *J C* 1969 *4* 709

GRUZE, J J Light and electron microscope observations on the gastric mucosa of the frog (Rana escu *Z. Zellforsch.* 1971 *117* 87 102.

HELANDER, H. F., S. S. SANDERS, W. S. REHM and B. I HIRSCHOWITZ, Quantitative aspects of gastri phology In G Sachs and E. Heinz (eds.) *Gastric secretion*, pp. 69–88. Academic Press, New York

KIDDER, G W and C. W MONTGOMERY CO diffusion into frog gastric mucosa as rate-limiting fa acid secretion. *Amer J Physiol.* 1974, *227* 300–304.

KIDDER, G W and W S. REHM, A model for the long time-constant transient voltage response to c epithelial tissues. *Biophys J* 1970, *10* 215–236.

NOYES, D H. and W S. REHM, Voltage response of the frog gastric mucosa to direct current. *Am Physiol.* 1970, *219* 184–192.

PERACCHIA, C. and B. S. MITTLER, New glutaraldehyde fixation procedures. *J Ultrastruct Res.* *39* 57–64.

REHM, W S., Acid secretion, resistance, short-circuit current and voltage-clamping in frog's sto *Amer J Physiol* 1962, *203*, 63–72.

[illegible] normal or slightly elevated level, *i.e.* autoregulation of the blood flow (for reference see [illegible]kow 1964). In the second major theory suggested by Thurau and his colleagues (Thurau [19]64, Thurau and Schnermann 1965, Schnermann *et al.* 1970) the sodium concentration [o]r the load) at the macula densa segment of the nephron was thought to be a determinant [o]f the renin release from the juxtamedullary cells, and thereby [vi]a the production of Angio[ten]sin II a determinant of the afferent vascular tone. This latter hypothesis has in recent [ye]ars been the objective of several investigations. The majority of these would seem to [su]pport the original suggestion at least when the sodium load was increased to super-normal [v]alues, whereas *a decreased load, on the contrary rarely produces the expected decrease in* [t]he afferent vascular tone. (See Schnermann *et al.* 1973.)

In the present paper the control of the afferent vascular tone as predicted by the myogenic [h]ypothesis has been examined. The transmural pressure has then been reduced by increasing [t]he renal interstitial pressure. The [v]ascular reactions with respect to the resistance pattern [w]ithin the superficial cortical vasculature has been analyzed via the study of the blood [f]low and the hydrostatic pressures within the different segments of the cortical vascular tree. The interstitial pressure was continuously monitored via about 50 µm thick PVC-catheters [p]ut under the renal capsula and placed into the subcapsular space.

Material and Methods

All experiments were carried out on male Sprague–Dawley rats weighing between 280–340 g. The animals were anesthetized with Inactin® (120 mg/kg) and placed on [a] servocontrolled heating pad. The left kidney [w]as exposed via [a] flank incision and suspended into [a] lucite cup. The different parameters were analyzed [wi]th different means according to

Micropuncture studies. For the study of intravascular and intratubular pressures, [a] servo controlled [mi]cro pressure device according to Wiederhielm (1964) as modified by Intaglietta (1970) was used. The [s]ystem was connected to sharpened glass capillaries with [a] tip diameter of [a]bout 4 µm. Glomerular capil[l]ary pressure was measured indirectly using the stop-flow technique according to Gertz (1966). [I]n this technique coloured mineral oil was first injected into an early proximal tubular segment until the filtration [ha]d ceased. The stop-flow pressure thus established [w]as measured with a second cannula inserted prox[im]ally to the first cannula. The glomerular capillary pressure was calculated by adding this pressure to the [so]-called oncotic pressure of the systemic plasma, the latter [w]as calculated from data on the protein concen[tra]tion in the plasma using the formulas given by Landis and Pappenheimer (1963). Effort was made t[o] [o]btain the early part of the proximal tubules. Randomly punctured tubules will have [a] stop flow pressur[e] of 3–5 mm Hg below the pressure in the most early part of the nephron (as investigated in some contr[ol] experiments). Only the latter result [wi]ll corroborate with the directly punctured glomeruli found occasionall[y] (ca. 5) on the kidney surface. The efferent arteriolar pressure was measured as the pressure within th[e] [s]tar vessels [e]asily identified on the kidney surface. The peritubular capillary pressure was obtained from randomly punctured small peritubular capillaries. Occasionally superficial small veins [we]re identifie[d] allowing [a] more complete evaluation of the pressure profile of the vascular network. Identification of th[e] structure punctured [w]as made by injection of Lissamine green or Evans blue through the same pipette [as] that used for the pressure measurements.

The glomerular filtration both with respect to single superficial nephron filtration as well as to tot[al] renal filtration [w]as analyzed using 125-Iodthalamate as the test substance. The amount of activity injecte[d] amounted to 100 µCi/h or less. The single glomerular filtration rate [w]as investigated on the same tubul[es] as those used for the determination of the stop-flow. For this purpose [a] small droplet of about 5 tubul[ar] lengths was injected, immediately followed by the suction of tubular fluid at [a] rate to keep the oil dropl[et] [in] [a] fixed position. The time of sampling was 2–3 min. During the sampling the intratubular pressure w[as] continuously monitored by the pressure pipette inserted proximal to the sampling pipette. Only tho[se] samplings in [wh]ich the pressure was normal or slightly decreased [w]as accepted. For the determination [of] the 125-I activity the sampling pipettes were put into tubes and counted in [a] gamma-detector. Plas[ma]

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Effect of Elevated Interstitial Pressure on the Renal Cortical Hemodynamics

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Abstract

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The influence of renal interstitial pressure on the resistance pattern within the superficial cortical ... has been investigated from determinations of 1) the glomerular blood flow w th a modified ... technique and 2) the intravascular hyd ostatic pressures.

Interstitial pressure was monitored a a 50 μm PVC-catheter placed into the subcapsular ... space. Two condit ons were analyzed *viz.* a) elevation of uretheral pressure t 70 mm Hg and b) ... stasis to 10–15 mm Hg. Both conditions produced an increase in the interstitial pressure from 1-2 ... to about 5 mm Hg as well as a ncreased hilar lymph flow nd protein flow of about the same ... vascular reactions were d fferent however. Uretheral stasis (but not the stasis of a single nephro... duced a decreased resistance in the afferent arterioles with concomitant increase i the pressures ... glomerular capillaries, and the peritubula capillary network. In contrast enous stasis produce... small changes n the parameters studied but fo the obvious rise in the peritubular capillary pressur...

The results suggest that factors other than the terst tial pressure are governing the afferent ... tone, the t bular wall tension might be one of these factors.

One of the more important characteristics of the renal blood flow refers to the a... of the kidneys to regulate their own blood supply when the perfusion pressure ... from about 70 mm Hg to 200 mm Hg, *i.e.* autoregulation. This autoregulative respons... been found to operate in intact kidneys in situ as well as in isolated kidneys perfused ... whole blood or plasma (*e.g.* Thurau and Kramer 1959 a, 1959 b, Ochwadt 1956, Waugh ... Waugh and Shanks 1960, Hinshaw 1964, Thurau and Henne 1964). The causative fa... however underlying this response remain largely unidentified. Two main hypotheses ... been suggested. In the classical myogenic hypothesis the transmural pressure difference ... the afferent arteriole is primarily thought to be the determinant of the vascular tone ... thereby the blood flow in the sense that an increased transmural pressure would lead t... arteriolar constriction and a decreased pressure to a vasodilatation. An increased bl... pressure would then increase the transmural pressure causing an increased afferent arter... resistance. This would result in a restitution of an initially increased blood flow back ...

mal or slightly elevated level, *i.e.* autoregulation of the blood flow (for reference see kow 1964). *In the second major theory suggested* by Thurau and his colleagues (Thurau 4, Thurau and Schnermann 1965 Schnermann *et al.* 1970) the sodium concentration (the load) at the macula densa segment of the nephron was *thought to be a determinant* the renin release from the juxtamedullary cells, and thereby via the production of Angiotensin II, a determinant of the afferent vascular tone. This latter hypothesis has in recent years been the objective of several investigations. The majority of these would seem to support the original suggestion at least when the sodium load was increased to super-normal levels, whereas a decreased load, on the contrary rarely produces the expected decrease in the afferent vascular tone. (See Schnermann *et al.* 1973.)

In the present paper *the control of the afferent vascular tone as predicted by the myogenic hypothesis* has been examined. The transmural pressure has then been reduced by increasing the renal interstitial pressure. The vascular reactions with respect to the resistance pattern within the superficial cortical vasculature has been analyzed via the study of the blood flow and the hydrostatic pressures within the different segments of the cortical vascular tree. The interstitial pressure was continuously monitored via about 50 μm thick PVC-catheters put under the renal capsule and placed into the subcapsular space.

Material and Methods

All experiments were carried out on male Sprague-Dawley rats weighing between 280–340 g. The animals were anesthetized with Inactin (120 mg/kg) and placed on a servocontrolled heating pad. The left kidney was exposed via a flank incision and suspended into a lucite cup. The different parameters were analyzed in different series according to:

Micropuncture studies. For the study of *intravascular and intratubular pressures*, a *servo controlled* [illegible] pressure device according to Wiederhielm (1964) as modified by Intaglietta (1970) was used. The system was connected to sharpened glass capillaries with a tip diameter of about 4 μm. Glomerular capillary pressure was measured indirectly using the stop-flow technique according to Gertz (1966). In this technique coloured mineral oil was first injected into an early proximal tubular segment until the filtration had ceased. The stop-flow pressure thus established was measured with a second *cannula inserted proximally* to the first cannula. The glomerular capillary pressure was calculated by adding this pressure to the colloid osmotic pressure of the systemic plasma, the latter was calculated from data on the protein concentration in the plasma using the formulas given by Landis and Pappenheimer (1963). Effort was made to utilize the early part of the proximal tubules. Randomly punctured tubules will have a stop flow pressure of 3–5 mm Hg below the pressure in the most early part of the nephron (*as investigated in some control experiments*). Only the latter result will corroborate with the directly punctured glomeruli found occasionally ([illegible] 3) on the kidney surface. The efferent arteriolar pressure was measured as the pressure within the star vessels easily identified on the kidney surface. The peritubular capillary pressure was obtained from randomly punctured small peritubular capillaries. Occasionally superficial small vessels were identified allowing a more complete evaluation *of the pressure profile of the vascular network. Identification of the* structures punctured was made by injection of Lissamine green or Evans blue through the same pipette as that used for the pressure measurements.

The glomerular *filtration* both with respect to single superficial nephron filtration as well as to total renal filtration was analyzed using 125-Iodothalamate as the test substance. The amount of activity injected amounted to 100 $\mu Ci/h$ or less. The *single glomerular filtration rate was investigated on the same tubules* as those used for the determination of the stop-flow. For this purpose a small droplet of about 5 tubular lengths was injected, immediately followed by the suction of tubular fluid at a rate to keep the oil droplet at a fixed position. The time of sampling was 2.5 min. During the sampling the intratubular pressure was continuously monitored by the pressure pipette inserted proximal to the sampling pipette. Only those samplings in which the pressure was *normal or slightly decreased was accepted. For the determination of* the 125-I activity the sampling pipettes were put into tubes and counted in a gamma-detector. Plasma

TABLE I Cardiac output total renal blood flow superficial and juxtamedullary glomerular blood f during control antidiuresis and during the elevation of the ureteral pressure (upper pa during the elevation of the renal venous pressure (lower panel). From the table it could the conditions induced will be accompanied by essentially no changes in the circula meters studied

Experimental condition	Cardiac output ml/min 100 g	Experimental kidney ml/min 100 g	Control kidney ml/min 100 g	Superficial glomeruli nl/min 100 g	Juxtamedullary glomeruli nl/min 100 g
Control, n = 13	27.2 ± 5.1	1.91 ± 0.16	1.92 ± 0.44	99.0 ± 29.4	69.0 ± 15.3
P = 20 mm Hg	27.3 ± 8.6	1.76 ± 0.50	1.90 ± 0.79	93.1 ± 28.0	73.3 ± 45.8
Control, n = 9	32.7 ± 5.0	2.30 ± 0.36	2.41 ± 0.40	129.5 ± 20.0	110.5 ± 36.2
P = 11.0 ± 0.8 mm Hg	6.7 ± 4.0	1.95 ± 0.10	2.15 ± 0.43	131.2 ± 21.7	104.8 ± 32.6

activity was determined from blood samples withdrawn during the middle of the sampling period. estimation of the total filtration, urine was sampled in 10–15 min periods.

The *interstitial pressure* was measured as the pressure within the subcapsular interstitial space same pressure measuri g device, but the glass capillary was replaced by a thin PVC-catheter diameter of less than 50 μm. The catheter was ntroduced through a hole in the renal capsule an in the subcapsular space about 1 mm from the hole. The hole was then sealed with a silicon rubb pound (Källskog and Wolgast 1973). These measurements were performed separately after the co of the micropuncture studies since the regist ation of two pressures (g capillary and interstit found to nterfere with each other

The *intrarenal blood flow* cond tions were nvest gated with the microsphere technique using 141-Co and 85-Sr labelled 3M microspheres (3M Co St Paul, Minn. USA). The spheres were fir with ultrasonic agitation and then separated by sedimentation in large glass cylinders as descri iously (Källskog *et al.* 1972). The size ra ge of these spheres could then be reduced to ± 2.65 μm and w th no aggregate among the injected spheres. 400 000 of each type of spheres, suspended of rat plasma, were injected approximately 20 s thro gh a catheter inserted into the right carot with is tip placed just above the aortic valves. During the injection blood was continuously from the femoral artery at a rate of 0.6 ml/min The blood flow of the kidneys and single glome analyzed as accordi g to Bankir *et al.* 1973

The *renal hilar lymph* was collected from small PE-catheters nserted into hilar lymph vessel. A lymph flow sampling conditions were considered to occur when the lymph essel was somewhat co proximal to the catheter's tip. The flow rate was determ ned from the length of the fluid column in c bore glass capillaries (microcaps 5.20 and 100 μl, Drummond Scientific Co., USA). The protein co tion in the plasma and lymph was analyzed according to Lowry (1951).

Two experimental conditions were tudied 1) elev ted ureteral pressure to 20 mm Hg and 2) renal venous pressure to 11 15 mm Hg. The latter condition was produced by constriction of th vei with a thin thread placed around the vessel the venous pressure was monitored via a steel inserted distally to the stricture.

Results

The results from the blood flow study during elevated ureteral pressure and elevated v pressure, respectively are shown in Table I The cardiac output was about 30 ml/m 100 g rat in all the conditions studied. The total renal blood flow was close to 2 ml/m 100 g rat and showed no differences between the experimental and the control ki Since the micropuncture data primarily refer to the superficial structures, the blood conditions in these superficial glomeruli are of special interest In these glomeruli the b flow is relatively large—estimated at about 100 nl/min per 100 g rat The juxtamedu

TABLE II. Proximal tubular "stop-flow pressure" and hydrostatic pressures within the superficial cortical vasculature, proximal tubules, subcapsular interstitial space together ith the data on single nephron and total kidney filtration and on the urine production during control and during venous either ureteral (upper panel) or renal venous (lower panel) stasis. For explanation see text.

Experimental condition	n	Stop-flow pressure mm Hg	Star-vessel pressure mm Hg	Capillary pressure mm Hg	Tubular pressure mm Hg	Total renal filtration ml/min 100 g	Single nephron filtration nl/min 100 g	Urine flow µl/min	Inter-stitial pressure mm Hg
Control,	6	32.8±1.8	13.0±1.4	11.1±1.4	13.3±1.7	0.32±0.04	14.3±1.1	5.1±2.6	1.3±0.7
20 mm Hg		40.3±2.5	18.9±1.8	16.3±2.0	22.3±1.8	0.35±0.10	16.2±1.5	5.6±4.4	4.0±1.6
Control,	8	35.5±5.2	15.3±1.7	12.5±2.1	14.8±1.4	0.49±0.08	21.4±1.3	6.6±2.4	1.0±0.3
14.8±3.9 cm Hg		33.9±4.6	20.6±3.6	17.6±2.8	19.5±4.2	0.34±0.05	15.2±2.9	8.2±0.8	5.0±2.5

lomeruli, here depicted for reference purposes, showed slightly lower values. From Table I t ould seem that the blood flow in all the structures studied will remain essentially unchanged during both the induced ureteral and enous stasis.

The micropuncture data from 6 rats subjected to ureteral pressure elevation and 8 rats ith venous pressure elevation are depicted in Table II. Single nephron and total glomerular iltration rates have been studied in 6 and 4 expts. in the two groups, respectively The alues obtained from one individual experiment were derived from 3–6 single determinations of pressures or determinations of a single nephron filtration. The scatter of these variables in the individual experiment was negligible the pressures in the peritubular capillary network, for instance, did not differ from each other more than 1 2 mm Hg. The differences from one experiment to the other were also ery slight which seems clear from the table. The interstitial pressures shown to the right in Table II were not measured in connection ith the other measurements, but estimated after the completion of the micropuncture studies.

Table II is utilized for the description of the pressure profile within the cortical superficial vascular network shown in Fig. 1 The control alues are in accordance with our own previous data as well as those of others (Andreucci *et al.* 1971 Brenner *et al.* 1972, Falchuk *et al.* 1971 a, 1971 b) and require therefore only few comments. The single nephron filtration rates are in these series relatively large (see Giebisch, Symposium on Renal Micropuncture Techniques (1972)), but in accordance with the likewise high superficial glomerular blood flow found in the study As the hematocrit in the rats used is slightly below 50 %, the plasma flow will amount to bout 50 nl/min, this should then mean a filtration fraction of somewhat less than 30 %, which would seem reasonable. It is somewhat puzzling, however that the filtration rates in the study with venous stasis were even higher This is most likely due to the higher blood flow as well as the somewhat higher glomerular capillary hydrostatic pressure found in this series The reason for this phenomenon remains unexplained. However the manipulation of the renal ein with the division of the vessel from the artery and the application of a thread for clamping may be contributing cause. Otherwise the rats were of identical size and were treated in the same way

Elevation of the ureteral and venous pressures to such an extent that the same increase in

TABLE I Cardiac output total renal blood flow superficial and juxtamedullary glomerular blood [illegible] during control antidiuresis and during the elevation of the ureteral pressure (upper panel) and during the elevation of the renal venous pressure (lower panel). From the table it would seem that the conditions induced will be accompanied by essentially no changes in the circulatory parameters studied

Experimental condition	Cardiac output ml/min 100 g	Experimental kidney ml/min 100 g	Control kidney ml/min 100 g	Superficial glomeruli nl/min 100 g	Juxtamedullary glomeruli nl/min 100 g	Weight g
Control, n = 13	27 2 ± 5 1	1 91 ± 0.16	1 92 ± 0 44	99 0 ± 29 4	69.0 ± 15 3	2[illegible] ± 4[illegible]
P = 20 mm Hg	27.3 ± 8 6	1 76 ± 0.50	1 90 ± 0.79	93 1 ± 28.0	71.5 ± 45 0	
Control, n = 9	32.7 ± 5 0	2.30 ± 0.36	2.41 ± 0.40	129.5 ± 20.0	110 5 ± 36 2	2[illegible] ± [illegible]
P = 11.0 ± 0 8 mm Hg	26.7 ± 4.0	1 95 ± 0.10	2.15 ± 0 43	131.2 ± 21 7	104.8 ± 32 6	

activity was determined from blood samples withdrawn during the middle of the sampling period. For the estimation of the total filtration urine was sampled in 10–15 min periods.

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The *intrarenal blood flow* conditions were investigated with the microsphere technique using 15 μm 141-Ce and 85-Sr labelled 3M microspheres (3M Co St Paul Minn. USA). The spheres were first treated with ultrasonic agitation and then separated by sedimentation in large glass cylinders as described previously (Källskog *et al.* 1972). The size range of these spheres could then be reduced to ±2 65 μm (+ S D) and with no aggregate among the injected spheres. 400 000 of each type of spheres, suspended in 0.5 ml of rat plasma, were injected in approximately 20 s through a catheter inserted into the right carotid artery with its tip placed just above the aortic valves. During the injection blood was continuously withdrawn from the femoral artery at a rate of 0.6 ml/min. The blood flow of the kidneys and single glomeruli was analyzed as according to Bankir *et al* 1973

The *renal hilar lymph* was collected from small PE-catheters inserted into a hilar lymph vessel. Accurate lymph flow sampling conditions were considered to occur when the lymph vessel was somewhat collapsed proximal to the catheter's tip. The flow rate was determined from the length of the fluid column in constant bore glass capillaries (microcaps 5.20 and 100 μl, Drummond Scientific Co., USA). The protein concentration in the plasma and lymph was analyzed according to Lowry (1951).

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The results from the blood flow study during elevated ureteral pressure and elevated venous pressure, respectively are shown in Table I The cardiac output was about 30 ml/min per 100 g rat in all the conditions studied. The total renal blood flow was close to 2 ml/min per 100 g rat and showed no differences between the experimental and the control kidney. Since the micropuncture data primarily refer to the superficial structures, the blood flow conditions in these superficial glomeruli are of special interest In these glomeruli the blood flow is relatively large—estimated at about 100 nl/min per 100 g rat. The juxtamedullary

II. Data on the renal hilar lymph flow and albumin flux after the induction of either elevated ureteral (upper panel) or elevated venous pressure. The table demonstrates that the conditions induced was accompanied with clear and stable rise in the hilar lymph flow and protein flux, whereas the lymph protein concentration shows a barely significant decrease. The fractional change corresponds to the increase in interstitial pressure found under the same conditions, which could be predicted if the interstitial pressure monitored was representative for the whole of the renal interstitium.

...mental ...on	Lymph flow µl/min	Protein concentration g	Protein flux µg/min
...ol, n 7	0.46 ± 0.24	1.7 ± 0.2	8 ± 5
...0 mm Hg	1.11 ± 0.82	1.4 ± 0.3	17 ± 16
...onal change	2.3 ± 0.7	0.8 ± 0.1*	1.9 ± 0.6
...ol, 4	1.8 ± 0.9	2.7 ± 1.8	50 ± 32
...0–15 mm Hg	3.8 ± 1.4	2.2 ± 0.3	86 ± 49
...onal change	2.2 ± 0.4	0.9 ± 0.2*	1.9 ± 0.7

...ly significant change (P 0.01).
...t significant change (P 0.10)

...sorption was decreased in this series. At control conditions 0.4% of the total filtration ...ured in the urine against the figure of 0.8% for the venous pressure elevation in spite ...hat the glomerular filtration had decreased.

...these two series the interstitial pressure was raised about 3 times greater than the control ...ssure. This ought to be followed by an increased lymph flow For this reason the lymph ...w and the lymph protein flux were investigated in connection with the study of the intra-...al blood flow distribution. The results are depicted in Table III The table shows that ...ring the conditions studied the lymph flow was increased, i.e. in accordance with the ...ected prediction, both from a qualitative and also approximately from a quantitative ...int of view The lymph flow increased with 215% and 330% during ureteral and venous ...mping, respectively The increased lymph flow was in both series accompanied by a slight ...crease in the lymph protein concentration the lymph protein flow was raised to 270% ...d 290% during ureteral and venous stasis, respectively

Discussion

...thodological

...here the pressure data is concerned some comments with respect to the measurements of ...e renal interstitial pressure should be made. The measurement of subcapsular interstitial ...ressure (hydrostatic and colloid-osmotic) was introduced by Wunderlich *et al.* in 1971 ...t was then argued that the subcapsular space from an anatomical point of view is a part of ...he renal interstitium. It was shown to be in free and rapid communication with the rest of ...he interstitium as indicated by the rapid appearance (about 20 min) of radioactive albumin ...n the hilar lymph after a single injection through a small PVC catheter inserted into the ...ubcapsular space. The time of the albumin transport equals the time of passage from the ...peritubular blood stream to the hilar lymph (Wolgast *et al.* 1973).

During cold artificial perfusion with solutions of different compositions the interstitial

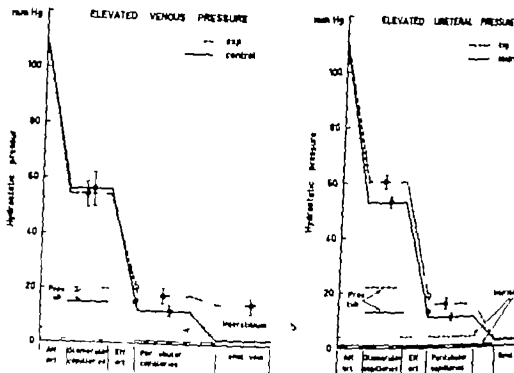


Fig. 1 Hydrostatic pressures within the superficial cortical vasculature, the proximal tubules and subcapsular interstitium (mean values ± S.D.) during control antidiuretic conditions and the elevated ureteral pressure (right panel) and during control antidiuresis and the elevation of the renal venous pressure (left panel). As the single superficial glomerular blood flow remained unchanged during the conditions studied, the figure suggests that ureteral stasis will be accompanied with a relaxation of the afferent arteriole with a concomitant pressure increase in the glomerular capillaries as well as in the peritubular capillary system. During venous stasis the glomerular capillary pressure was unchanged.

the interstitial pressure would occur produced clearly different patterns in the two groups studied. After the *ureteral pressure elevation* the glomerular capillary hydrostatic pressure was significantly raised. This combined with the fact that the blood flow was unaltered would mean a relaxation of the afferent arteriole. *The driving force for glomerular filtration* in the proximal end of the glomerulum (stop flow pressure minus proximal tubular pressure) in this series was estimated at 19.5 mm Hg at control conditions, decreasing to 18.0 mm Hg during the increased ureteral pressure, *i.e.* a *negligible change. The single glomerular filtration rate* showed a small but insignificant increase. The total filtration showed the same appearance with a slight change from 0.32 ml/min to 0.35 ml/min 100 g. The urine production showed an increase of about the same size which then means that the tubular fractional reabsorption also remained unchanged.

The phenomena described are restricted to uretheral stasis *i.e.* stasis of all the nephrons. Blockade of one single nephron (by distal oil blocks) was not accompanied by these reactions.

During *venous pressure elevation* the pressure drop over the glomerular capillary membranes showed a clear decrease from 20.7 to 14.4 mm Hg. This was accompanied by a decrease in the single nephron filtration rate of about the same magnitude. The total filtration rate showed the same pattern with a decrease from 0.49 ml/min to 0.34 ml/min 100 g. It is should again be noted that the figure of 0.49 ml/min 100 g is larger than the corresponding value found during the control condition in the series with ureteral clamping. Fractional

ring venous stasis the tubular pressure increased as a consequence of the increased inter stitial pressure. The tubular transmural pressure however will be different—increased uring ureteral and decreased during venous stasis. If this increased *tubular wall tension* ould be a determinant of the afferent arteriolar tone remains unclear.

The experimental conditions analyzed in the present work have previously been the objec t of an intensive study. Most of the earlier investigations deal with determinations of the renal vascular resistance as analyzed from data on the total renal blood flow and the lomerular filtration rate. In the majority of these studies a more or less pronounced increase n the total renal blood flow have been noticed during the elevation of ureteral pressure Gilmore 1964, Selkurt 1963, Thurau 1964). The subject has more recently been studied by Gil *et al.* (1969) in which increased tubular pressure was induced by a urinary obstruction r by a large urine flow. In these studies the increase in the total renal blood flow on changing he ureteral pressure was attributed to a decreased afferent arteriolar tone. This in turn was hought to be due to an increased interstitial pressure *producing a decreased transmural* ressure difference. During elevation of the renal venous pressure only slight changes in the renal vascular resistance will occur (Haddy *et al.* 1958).

Where micropuncture studies are concerned a clear increase in the peritubular capillary and the proximal tubular pressures has been found both as a consequence of the elevated ureteral and venous pressures (Gottschalk and Mylle 1956, Allison *et al.* 1972). These investigations also showed that the peritubular capillary pressure will raise in parallel with the renal venous pressure but firstly when the latter pressure equals the normal pressure within the peritubular network, *i.e.* at about 10 mm Hg. Likewise Brenner *et al.* (1972) showed that tubular *stop-flow in an isolated proximal tubular segment* will increase with an increasing ureteral pressure, a phenomenon compatible with the increased glomerular capillary hydrostatic pressure as discussed here. To summarize, it can be stated that even though a more detailed description now is available for the reactions that occur during the actual experimental conditions, an acceptable explanation is still lacking.

References

ANDREUCCI, V. E., J. HERRERA-ACOSTA, F. C. RECTOR, Jr and D. W. SELDIN, Effective glomerular filtration pressure and single nephron filtration rate during hydropenia, elevated ureteral pressure, and acute volume expansion with isotonic saline. *J. clin. Invest.* 1971. *50.* 2230–2234.

ALLISON, M. E. M., E. M. LIPHAM and C. W. GOTTSCHALK, Hydrostatic pressure in the rat kidney. *Amer. J. Physiol.* 1972. *223.* 975–983.

BANKIR, L., N. FARMAN and J. P. GRÜNFELD, Radioactive microsphere distribution and single glomerular blood flow in the normal rabbit kidney. *Pflügers Arch. ges. Physiol.* 1973. *342.* 111–123.

BRENNER, B. M., J. L. TROY and T. M. DAUGHARTY. Pressures in cortical structures of the rat kidney. *Amer. J. Physiol.* 1972. *222.* 246–251.

FALCHUK, K. H. and R. W. BERLINER, Hydrostatic pressures in peritubular capillaries and tubules in the rat kidney. *Amer. J. Physiol.* 1971. *220.* 1422–1426.

FALCHUK, K. H., B. M. BRENNER, M. TADOKORO and R. W. BERLINER, Oncotic and hydrostatic pressures in peritubular capillaries and fluid reabsorption by proximal tubule. *Amer. J. Physiol.* 1971 b. *220.* 1427–1433.

FOLKOW, B. Description of the myogenic hypothesis. *Circulat. Res.* 1964. *14–15.* 279–287. Suppl. I.

FRÖSEN, L., A. ENGBERG, Ö. KÄLLSKOG and M. WOLGAST. Renal transplantation in the rat. IV. Cortical pressure gradients measured in vivo and during isolated perfusion with reference to renal function after transplantation. *Scand. J. clin. Lab. Invest.* 1975. *35.* 463–472.

pressure was found to be inversely related to the colloid-osmotic pressure of the per used, reaching values up to 30 mm Hg during the perfusion with colloid-free so (Frödin *et al* 1975). Negative interstitial pressures could also be obtained when sol with very high colloid osmotic pressures (10% albumin) and low perfusion rates wer Furthermore, the increase in perfusion pressure during colloid-free perfusion compa that during 5% albumin perfusion on these cold "passive kidneys" could be related increase in the interstitial pressure. This phenomenon could be predicted to occur measured interstitial pressure was representative for the whole of the renal interstitium arguments referred to thus suggest that the present measurements are valid. Previous s tions in which so-called needle-pressure was used as the interstitial pressure would unlikely due to the simple fact that these pressures equal the proximal tubular pressur thereby are considerably higher than the distal tubular pressure (about 5 mm Hg).

Physiological

The conditions studied have in common that the interstitial pressure was raised to the same extent A further increase in the ureteral and the venous pressures (the latte been made in the pilot series preceding the present study), showed a further increa interstitial pressure. However even if the interstitial pressure changed to the same de the vascular reactions were completely different. This strongly indicates that the inter pressure *per se* is not a determinant of the afferent arteriolar tone.

During *venous pressure elevation* the primary event would seem to be an increase o peritubular capillary pressure The increase is, however less pronounced than the inc in the venous pressure. The pressure drop between the peritubular capillaries and the r vein will thereby vanish The localization of the resistance responsible for the steep pre drop between the peritubular capillaries and the renal vein during normal condition mains unclear

The glomerular capillary pressure in this series is not changed, which then means that pressure drop and also the resistance over the efferent arteriolae was decreased—since blood flow was unchanged In contrast afferent arteriolar tone remained essentially changed. The proximal tubular pressure was elevated producing a decreased driving fo for glomerular filtration and consequently a concomitant decrease in the amount of f filtered. The cause for the pressure elevation in the tubules is obviously due to the ra interstitial pressure compressing the tubules, *i.e* a passive increase.

In the series on *ureteral stasis* the *primary* change is the increase in the tubular press The possibility now exists that the tubules compress the interstitium between the tub wall and the stiff renal capsula. This would however only lead to a transient increase in interstitial pressure and only a transiently increased renal lymph flow This explanation the increased interstitial pressure can therefore be ruled out Instead the interstitial press raise is produced by the increased peritubular capillary pressure which in turn is due to decreased afferent arteriolar tone. The outflow resistance, which in the case of the veno pressure elevation was decreased will under these conditions be somewhat increased.

The two conditions analyzed now share another change in the parameters studied, *i.e.* increased proximal tubular pressure During ureteral stasis the increase is obvious where

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By

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It has been proposed that a sulphomucopolysaccharide-protein (SMPS-prot) complex may be involved in the binding of catecholamines (CA) within vesicles of various tissues (Uvnäs 1973). However it seems to be still a matter of controversial opinions; some workers finding evidence for such an involvement (Fillion, Nosal and Uvnäs 1971 Åborg *et al.* 1972, Margolis and Margolis 1973 Margolis, Jaanus and Margolis 1973) while others have reported and interpreted results to the contrary (Da Prada, von Berlepsch and Pletscher 1972). The main tissue that has been subjected to investigation appears to be the adrenal medulla (Fillion, Nosal and Uvnäs 1971 Margolis and Margolis 1973 Margolis, Jaanus and Margolis 1973) while little reference is made to the storage of CA within the central nervous system. The distribution of sulphomucopolysaccharides (SMPSs) among subcellular fractions has been investigated only in preparations from the whole brain (Vos *et al.* 1969 Kariyama and Okada 1971 Bacchawat *et al.* 1972).

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GERTZ, K. H J A. MANGOS, G BRAUN, and H D. PAGEL, Pressure in the glomerular capillaries of the rat kidney and its relation to arterial blood pressure. *Pflügers Arch. ges. Physiol.* 1966. 288. 369-3
GILMORE, J P Renal vascular resistance during elevated ureteral pressure. *Circulat Res.* 1964. 148–152. Suppl. I
GOTTSCHALK, C. W and M MYLLE, Micropuncture study of pressures in proximal tubules and peritubular capillaries of the rat kidney and their relation to ureteral and venous pressures. *Amer J Physiol.* 185 430–439
HADDY F J J SCOTT M FLEISHMAN and D EMANUEL, Effect of change in renal venous pressure on renal vascular resistance, urine and lymph flow rates. *Amer J Physiol.* 1958. 195 97 110.
HINSHAW L. B., Mechanism of renal autoregulation Role of tissue pressure and description of a factor hypothesis. *Circulat Res* 1964. 14–15 120–132. Suppl. I
INTAGLIETTA, M R. F PAWULA and W R. TOMPKINS, Pressure measurements in the mammalian vasculature. *Microvasc R s.* 1970 2. 212 220.
KÄLLSKOG, Ö and M WOLGAST Driving forces over the peritubular capillary membrane in the rat kidney during antidiuresis and saline expansion. *Act physiol. scand.* 1973 89 116–125.
KÄLLSKOG, Ö H. R ULFENDAHL and M WOLGAST Single glomerular blood flow as measured with carbonized 141-Ce labelled microspheres. *Acta physiol scand.* 1972. 85 408–413.
KIIL, F J KJEKSHUS and E. LOYNING Role of autoregulation in maintaining glomerular filtration at large urine flow *Acta physiol scand.* 1969 76. 24–39
LANDIS, E. M and J R PAPPENHEIMER, Exchange of substances through the capillary walls. *Handbook Physiology Circulation II* Waverly Press, USA. 1963 961 1034.
LOWRY O H N J ROSEBROUGH A. L. FARR and R. J RANDALL, Protein measurement with the phenol reagent *J biol Chem.* 1951 193 265–275.
OCHWADT B., Zur Selbststeuerung des Nierenkreislaufes. *Pflügers Arch ges. Physiol.* 1956. 262. 2
Renal Micropuncture Techniques A Symposium. Ed. G H Giebisch, *Yale J Biol. Med.* 1972. 45.
SCHNERMANN, J A. E. G PERSSON and B. ÅGERUP Tubuloglomerular feedback. Nonlinear relation between glomerular hydrostatic pressure and loop of henle perfusion rate. *J clin. Invest* 1973. 52 869
SCHNERMANN, J F S. WRIGHT J M DAVIS, W v S ACKELBERG and G GRILL, Regulation of superficial nephron filtration rate by tubulo-glomerular feedback. *Pflügers Arch. ges. Physiol.* 197 147 175
SELKURT E. E., Effect of ureteral blockade on renal blood flow and urinary concentrating ability *J Physiol.* 1963 205 286–292.
THURAU, K Renal hemodynamics. *Amer J Med.* 1964. 36. 698–719
THURAU K. and G HENNE, Die transmurale Druckdifferenz der Widerstandsgefässe als Parameter der Widerstandsregulation der Niere. *Pflügers A h. ges Physiol* 1964 279 156–177
THURAU K and K KRAMER, Die Reaktionsweise der glatten Muskulatur der Nierengefässe auf Dehnungsreize und ihre Bedeutung für die Autoregulation des Nierenkreislaufes. *Pflügers Arch. ges.* 1959 a. 268 188–203
THURAU K. and K. KRAMER, Weitere Untersuchungen zur myogenen Natur der Autoregulation des Nierenkreislaufes. *Pflügers Arch ges. Physiol* 1959 b. 269 77 93.
THURAU K. and J SCHNERMANN, Die Natriumkonzentration an den Macula-densa Zellen als regulierender Faktor für das Glomerulumfiltrat (Mikropunktionsversuche). *Klin Wschr* 1965. 43 410–413.
WAUGH W H Myogenic nature of autoregulation of renal flow in the absence of blood corpuscles. *Circulat Res.* 1958 6. 363–372.
WAUGH, W H and R. G SHANKS, Cause of genuine autoregulation of the renal circulation. *Circulat Res.* 1960. 8 871–888.
WIEDERHIELM, C A., J W WOODBURY S. KIRK and R F RUSHMER, Pulsatile pressures in the microcirculation of frog's mesentary *Amer J Physiol.* 1964 207 173–176.
WOLGAST M H R. ULFENDAHL, Ö KÄLLSKOG N RASMUSSEN J L. ATKINS and G G PINTER, Pressure and protein distribution in kidney. *Proteins of the biological fluids—21st colloquium.* Ed. H. Peeters. Pergamon Press, Oxford and New York. 1973 413–417
WUNDERLICH P E. PERSSON J SCHNERMANN, H R ULFENDAHL and M. WOLGAST Hydrostatic pressure in the subcapsular interstitial space of rat and dog kidney *Pflügers Arch. ges. Physiol.* 1971 328. 3

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nerve terminals produced by homogenisation of brain tissue—as functional units from discrete areas of the rat brain (for reviews, see Whittaker 1969). Th been combined with the effect of chemical denervation with 6 hydroxydopar DA) to investigate the possible relationship between CA and ^{35}S-labelled cor sociated with the monoamine-storing sites. An attempt to characterize and iden phur-containing compounds has been made.

Methods and Materials

Albino rats (Sprague Dawley) were used in the experiments. For chemical denervation of the neurotoxic compound 6-OH DA (3 100 mg/kg, dissolved in saline containing 0.2 mg/ml as was administered subcutaneously to newborn rats in a volume of 0.05 ml. The initial dose within 4 h after birth and the subsequent 2 doses on the following days at 4 h intervals. Cont received a similar njection sequence of saline. All animals were sacrificed by cervical disloc light chloroform anesthesia, at the age of 10–14 weeks. Radioactive sodium sulphate ($Na_2{}^{35}$S free, Kjeller Norway) was administered subcutaneously in a dose of 8 mCi/kg 24 h before sacr

Preparation f ynaptosomes

Several brain regions (cerebral cortex, cerebellum, hypothalamus and pons) were dissected out and homogenized in 10 olumes of 0.25 M sucrose usi g a glass homogenizer with teflon pe at a speed of bout 1 000 rpm. The homogenates were centrifuged at 600 g for 10 min and th tant layer used as the crude synaptosome preparation fo incubation studies.

In vitro incubation f ynaptosomes

Aliquots (100 μl) f the supernatant layer were incubated in Krebs-Ringer bicarbonate to lach H noradrenaline (^{3}H NA) was added n rder t determine the amount of amine taken up (fi see Jonsson t al 1974). After 5 min incubation period at 37°C (Sachs and Jonsson 1972) the rea terminated by the addition of 8 ml cold Krebs-Ringer buffer and the mixture centrifuged at 1 for 10 min to produce a synaptosomal pellet The pellet was dissol ed in 1 ml 1 N NaOH, thereaft ethanol-toluene scintillation solution was added and the H and ^{35}S radioactivity determined. The uptake at 0 C, which can be considered as extraneuronal ptake, has been btracted from the up ues obtained at +37°C (= active uptak).

G adient centrifugation

In other experiments a pre-incubated ynaptosomal fraction was layered on a linear continuous density gradient (3.5 ml) ranging from 0.3–1 6 M sucrose, as previously described (Jonsson et al Following centrifugation at 75 000 g for 2 h the t bes were pierced at the bottom and bout 25 fr collected. After addition of 10 ml toluene phosphor Triton X 100 mixture (4 1) the radioactivity H and ^{35}S was determined

Lysis of synaptosomes

I some experiments the synaptosomal pellet after labell g with H-NA, was taken and resuspe 1 ml distilled water to lyse the subcell lar particles After allowing the suspension t stand at room perature for 1 h, the t bes were centrif ged at 50 000 g f 15 min. The mounts of radioactiv and H NA present in the pellet or supernatant component were determined.

Identification f ^{35}S-labelled ompounds

) *Isolation and purification of SMPSs* Crude ynaptosomal pellets, some of them lysed, were dri ame o ernight, e tracted w th chloroform-methanol and the extract examined with thin layer chro graphy for presence of sulphatides. The extracted pellet was subjected to protein hydrolysis ith pa according t Olsson et al. (1968), followed by precipitation of the residual protein w th trichloroa acid. From the dialyzed supernatant layer the SMPS were precipitated w th 5% potassium acetat ethanol according to S epca and an Robertson (1967) The pernatant layer was examined by c matography on Sephadex G-50 according t M rgolis nd Margolis (1970) for presence of glycoprot Aliquots of all isolation steps were taken f measurement of ^{35}S radioactiv ty

) Separation and determination of SMPSs. 2 different separation procedures were carried out on the ...PSs precipitates: microelectrophoresis on cellulose acetate strips according to Wessler (1968) and frac...ation on cellulose columns according to Svejcar and ... Robertson (1967), both before and after ...atment with chondroitinase AC. Pieces of the electrophoretic strips, resp. aliquots of the column eluates ...e taken for measurement of ^{35}S-radioactivity. The strips were dissolved in acetone which was then ...porated. 10 ml Instagel (Packard) was added to the samples and the ^{35}S radioactivity measured.

...asurement of radioactivity

...Packard liquid scintillation spectrometer 3375 was used. All results were corrected for quenching by ... of the external standard system. In case of simultaneous labelling with ^{3}H and ^{35}S double-channel ...ique was applied and corrections made for quenching and for spillover between the channels.

Results

...H-NA uptake and synaptosomal ^{35}S content

...ntrol rats and rats treated at birth with 6-OH DA were sacrificed in the adult age, 24 h ...lowing an injection of ^{35}S-labelled sodium sulphate. Pretreatment at birth caused a mark... decrease in the *in vitro* uptake of ^{3}H NA into synaptosomes from the cerebral cortex, ...ing about 20% of control values, while the uptake in the hypothalamic synaptosomes ...s somewhat less affected, 70% of control (Fig. 1). However ^{3}H NA uptake in synapto...mes isolated from the pons-medulla region showed a considerable increase, being about ...0% of control value (Fig. 1). A comparison of the ^{35}S content of each synaptosomal pre...ration showed also a decrease in the cortical and hypothalamic regions isolated from rats ...eated with 6-OH DA at birth to the order of 65-70% of control values, whereas no change ...as detected in the pons-medulla region (Fig. 1).

...radient centrifugation studies

...n sucrose gradient centrifugation studies, the ^{3}H NA-labelled synaptosomes from control ...imals showed similar equilibrium characteristics as the distribution of ^{35}S on the gradient

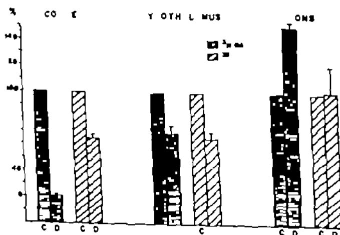


Fig. 1. *In vitro* uptake of ^{3}H-NA and radioactive ^{35}S-content of synaptosomes isolated from various regions of the brain of control rats (C) and denervated animals (D) treated at birth with 6-OH DA. The animal were sacrificed at the adult age. Each column represents the mean ± S.E. of 3 determinations and the values are expressed as percentages of control.

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In some experiments the synaptosomal pellet after labell ng w th H NA, was taken nd res 1 ml distilled ater to lyse the subcellular particles After allow ng the suspension to stand at perature for 1 h, the tubes were centrif ged at 50 000 g for 15 min. The amou t of radioac and H NA present in the pellet or supernatant component were determined.

Ident fication of ^{35}S-labelled compounds

a) *Isolation and purif ation of SMPSs.* Crude synaptosomal pellets, some of them lysed, *vacuo* overnight, extracted w th chloroform-methanol and the extract examined w th thin layer graphy for presence of sulphatides. The extracted pellet was subjected to protein hydrolysis according t Olsson *et al.* (1968), followed by precipitation of the residual protein w th tric acid From the dialyzed supernatant layer the SMPS were precipitated with 5 potassium ethanol according to Svejcar and an R bertson (1967) The supernatant layer was examine matography on Sephadex G 50 accordi g to Margolis and M rgolis (1970) for presence of gly Al quots of all isolation steps were taken for measurement of ^{35}S radioact ty

. Percentage release of radioactive sulphur and tritium into soluble fractions of synaptosomes from different brain regions following lysis in distilled water. Each figure represents the mean ±S.E. of 4 expts.

Region	Released	
	^{35}S	^{3}H-NA
x	17.3 ± 3.3	79.1 ± 3.5
thalamus	21.7 ± 6.9	85.6 ± 2.9
ellum	21.7 ± 4.4	81.9 ± 4.4
	14.5 ± 3.2	90.6 ± 3.6

. 2). Synaptosomes from cortex, cerebellum and hypothalamus showed a peak equi-ting at 0.9–1.2 M sucrose. The hypothalamus also yielded a smaller secondary peak at 0.7 M sucrose. A similar distribution pattern was observed for ^{35}S.

is of synaptosomes

llowing lysis, the synaptosomes from the cortex, hypothalamus and cerebellum lost out 20% of their ^{35}S content which appeared in the supernatant layer. The percentage soluble ^{35}S in the pons was slightly lower, about 15% (Table I).

ncorporation patterns of ^{35}S-incorporation into synaptosomes

adioactive sulphur was found to be incorporated in several types of compounds isolated ring fractionation of the crude synaptosomal pellet (Table II). The cortex, hypothalamus d cerebellum show a very similar pattern, roughly one third is represented by lipid-bound S, one third by sulphomucopolysaccharide-bound and about one third was recovered in e supernatant layer after SMPS precipitation. 3–4% remained in the residual protein pre-pitate, probably partly due to adsorption and partly to incomplete breakdown of the MPS-protein linkage. In the synaptosomal pellet isolated from the pons the prevalent rt of ^{35}S was found incorporated in the lipid fraction.

ualitative characterization of the ^{35}S-labelled compounds

he SMPSs isolated from synaptosomes of different brain regions showed essentially the me electrophoretic pattern. On the barium acetate electrophoreogram 2 maxima of ^{35}S adioactivity were detected. The Rf of one of them corresponded to the Rf of the reference eparin sulphate, the other peak lay between the reference compounds of dermatan sul-hate and chondroitin sulphate, thus pointing to the possible presence of a dermatan ulphate-chondroitin sulphate hybrid. The result of the separation of the SMPSs on cellu-ose columns is represented in Fig. 3. On fractionation of the SMPS on cellulose columns

. Distribution of radioactivity in synaptosomes (labelled in vivo with ^{3}H-NA) from a) cerebral cortex, b) hypothalamus and c) cerebellum in continuous sucrose density gradients ranging from 0.3–1.6 M su-rose. The gradients were centrifuged at 75 000 g for 120 min. Animals were injected 24 h previously with $Na_2{}^{35}SO_4$ (——) depicts tritium distribution and ( ) ^{35}S distribution. Representative diagrams have een chosen from 3–5 expts.

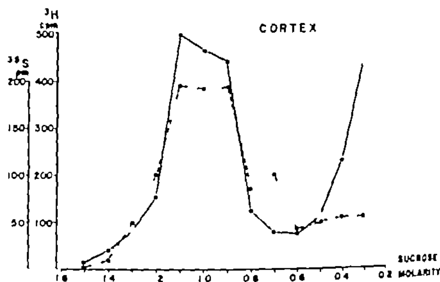


Fig. 2a.

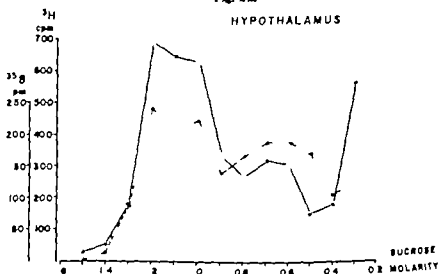


Fig. 2b.

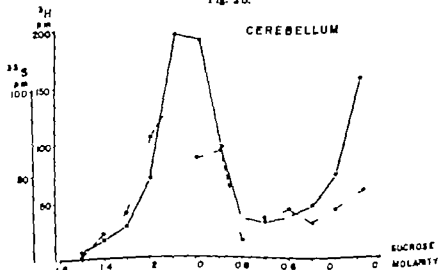


Fig. 2c.

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…gion	Released	
	³⁵S	H-NA
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…llum	23.7±4.4	81.9±4.4
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TABLE II Percentage distribution of radioactive sulphur among different groups of compound from synaptosomes of different brain regions. Precipitate = residual protein precip papain digestion Supernatant = supernatant layer after SMPS precipitation. The res mean ± S.E. of 3–4 expts.

Brain region	Radioactive sulphur ^{35}S %			
	Lipid-bound	Non-lipid bound		
		Precipitate	Supernatant	SMPS
Cortex	26.2 ± 1 7	4 3 ± 0.4	34.5 ± 0.7	34.8 ± 1.3
Hypothalamus	41 6 ± 4 9	3 1 ± 0.1	22.9 ± 2.7	32.3 ± 2.2
Cerebellum	38 4 ± 2.4	4 1 ± 0.2	26.6 ± 1.2	30.9 ± 1.2
Pons	58.4 ± 2.1	2.8 ± 0.02	16.9 ± 0.5	21 8 ± 1 9

according to Svejcar and van Robertson where the different SMPSs are eluted fro column in a certain order one maximum of ^{35}S radioactivity occurred in the fourt fifth fraction corresponding to chondroitin-4-sulphate, resp. chondroitin-6-sulphate another peak in the third fraction corresponding to heparan sulphate (Fig. 3). S from all four brain regions showed essentially the same fractionation pattern (Fig. 3 difference was demonstrated by fractionating SMPSs isolated from lysed and non- synaptosomal pellets. The high amounts of ^{35}S-labelled material sometimes appear the first fraction indicated in all probability breakdown products. When the SMPS cipitate was treated before fractionation with chondroitinase AC the amount of ^{35}S-lab compounds in the fourth and fifth fractions significantly diminished while it was c spondingly increased in the first fraction. For precise characterization of the chondr sulphate it would be necessary to use treatment with different chondroitinases because elution pattern of chondroitin sulphates can be slightly shifted depending on the molec weight and degree of hybridization Since, because of lack of material only chondroiti AC, slightly contaminated with chondroitinase B, was used, it was not possible to dis guish with certainty between chondroitin-4-sulphate, chondroitin-6-sulphate and derma sulphate.

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Discussion

The significant decrease in the uptake of H NA in synaptosomal fractions of cortex and hypothalamus from animals pretreated at birth with 6-OH DA has been interpreted as a

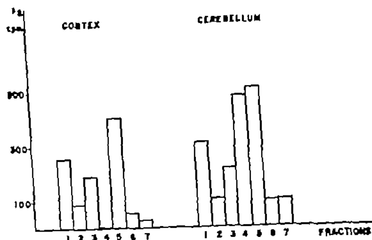


Fig. 3

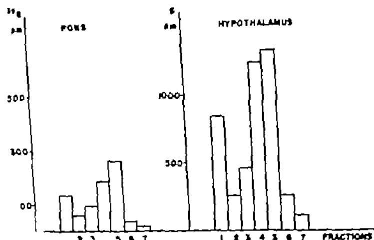


Fig. 3b.

Fig. 3. Fractionation of ^{35}S-labelled SMPS from different regions of rat brain on cellulose columns eluted with solvents of rising ionic strength. Order of elution. F – keratan sulphate or breakdown products of higher polymerized SMPS. F hyaluronic acid. F heparan sulphate. F – chondroitin-4-sulphate. F chondroitin-6-sulphate. F dermatan sulphate. F heparin. Representative diagrams have been chosen from 3–5 expts.

permanent and selective destruction of noradrenergic nerve terminals in these regions of the brain (Sachs and Jonsson 1972, Singh and de Champlain 1972). A reasonable suggestion to this phenomenon is that the dorsal ascending NA bundle, originating from the locus coeruleus cell group in the pons and supplying nerve terminals to the cerebral cortex, is preferentially attacked by 6-OH DA treatment (Jonsson *et al.* 1974). Associated with this effect is also dramatic increase in ^{3}H-NA uptake in the pons-medulla region. It has been suggested that an increased number of nerve terminals, due to an increased outgrowth from

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Cortex	26.2 ± 1.7	4.3 ± 0.4	34.5 ± 0.7	34.8 ± 1.3
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Cerebellum	38.4 ± 2.4	4.1 ± 0.2	26.6 ± 1.2	30.9 ± 1.2
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according to Svejcar and van Robertson where the different SMPSs are eluted from column in a certain order one maximum of ^{35}S radioactivity occurred in the fourth fifth fraction, corresponding to chondroitin-4-sulphate, resp. chondroitin-6-sulphate, another peak in the third fraction corresponding to heparan sulphate (Fig. 3). SM from all four brain regions showed essentially the same fractionation pattern (Fig. 3). difference was demonstrated by fractionating SMPSs isolated from lysed and non-l synaptosomal pellets. The high amounts of ^{35}S-labelled material sometimes appearin the first fraction indicated in all probability breakdown products. When the SMPS cipitate was treated before fractionation with chondroitinase AC the amount of ^{35}S-lab compounds in the fourth and fifth fractions significantly diminished while it was co spondingly increased in the first fraction For precise characterization of the chondr sulphate it would be necessary to use treatment with different chondroitinases because elution pattern of chondroitin sulphates can be slightly shifted depending on the molec weight and degree of hybridization Since, because of lack of material only chondroiti AC, slightly contaminated with chondrotinase B, was used it was not possible to di guish with certainty between chondroitin-4-sulphate, chondroitin-6-sulphate and derm sulphate.

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Discussion

The significant decrease in the uptake of H NA in synaptosomal fractions of cortex hypothalamus from animals pretreated at birth with 6-OH DA has been interpreted

...the whole brain (Margolis and Margolis 1970, for references see Balasubramanian and Bachhawat 1970).

In all four regions the SMPSs have been identified as chondroitin sulphate and heparan sulphate, in agreement with the results of Bacchawat *et al.* (1972) who identified these compounds in a synaptosomal preparation from the whole brain of the rat. The confirmation of identical SMPSs in synaptosomes from different regions seems to indicate a common feature in the composition of brain nerve endings, the SMPSs occur probably in a protein-bound form, as has been generally established for tissue SMPSs (for references see Brimacombe 1964, Rapoport 1969 Rodén 1967). In addition, Vos, Kuriyama and Roberts (1969) found the SMPS mainly located in synaptosomal vesicles isolated from whole brain preparations.

The amounts of ^{35}S-containing material obtained from the different brain regions were insufficient for quantitative determination of the SMPS or a closer characterization of their protein moieties probably present. We refrain therefore from any discussion about their possible amine binding abilities. We refer in this respect to two papers presently under preparation concerning the amine storing capacity *in vitro* of a protamine-heparin complex and of an ATP-free material from bovine medullary granules (Uvnäs and Åborg 1975).

...financial support from Swedish Medical Research Council (project No. B75-04X 39-11A) and from K.I. ...is gratefully acknowledged

References

...ABORG, C. H., G. FILLION, R. NORAL and B. UVNÄS, A sulphomucopolysaccharide-protein complex in the microvesicle fraction from nerves and tissue. *Acta physiol. scand.* 1972, 86, 427–429.
...BACHHAWAT, B. K., K. A. BALASUBRAMANIAN, A. S. BALASUBRAMANIAN, M. SINGH, E. GEORGE and E. V. CHANDRASEKARAN, Chemistry and metabolism of glycosaminoglycans of the nervous system. *Advanc. exp. Med. Biol.* 1972, 25, 51–71.
...BALASUBRAMANIAN, A. S. and B. K. BACHHAWAT, Sulfate metabolism in brain. *Brain Res.* 1970, 20, 341–360.
BRIMACOMBE, J. S. and J. M. WEBBER, *Mucopolysaccharides*. Elsevier Publ. Comp. 1964.
DA PRADA, M., K. VON BERLEPSCH and A. PLETSCHER, Storage of biogenic amines in blood platelets and adrenal medulla. *Naunyn-Schmiedebergs Arch. Pharmacol.* 1972, 275, 315–322.
FILLION, G., R. NORAL and B. UVNÄS, The presence of ... mucopolysaccharide-protein complex in adrenal medullary cell granules. *Acta physiol. scand.* 1971, 83, 286–288.
...JONSSON, G., CH. PYCOCK, K. FUXE and CH. SACHS, Changes in the development of central noradrenaline neurons following neonatal administration of 6-hydroxydopamine. *J. Neurochem.* 1974, 22, 419–426.
...KIYAMA, K. and T. A. OKADA, Incorporation of ^{35}S-sulphate into developing mouse brain, subcellular fractionation and electron microscopic studies. *Exp. Neurol.* 1971, 30, 18–29.
MARGOLIS, R. K. and R. U. MARGOLIS, Sulphated glycopeptides from rat brain glycoproteins. *Biochemistry* 1970, 9, 4389–4396.
MARGOLIS, R. U. and R. K. MARGOLIS, Isolation of chondroitin sulphate and glycopeptides from chromaffin granules of adrenal medulla. *Biochem. Pharmacol.* 1973, 22, 2195–2197.
MARGOLIS, R. K., S. D. JAANUS and R. U. MARGOLIS, Stimulation by acetylcholine of sulphated mucopolysaccharide release from the perfused cat adrenal gland. *Mol. Pharmacol.* 1973, 9, 590–594.
OLSSON, I., S. GARDELL and S. THUNELL, Biosynthesis of glycosaminoglycans in human leucocytes. *Biochim. Biophys. Acta* (Amst.) 1968, 165, 309–323.
PYCOCK, C. and G. JONSSON, Transmitter-dependent changes of equilibrium characteristics of catecholamine synaptosomes in sucrose density gradients. *Med. Biol.* 1974, 52, 260–268.
RAPOPORT, S. M., *Medizinische Biochemie*. VEB Verlag Volk und Gesundheit, Berlin 1969, p. 684–696.
RODÉN, L., Structure of mucopolysaccharide-protein complexes. *Abstracts from The Seventh Int. Congress of Biochemistry*, Tokyo 1967, Vol. 1, 69–70.

the locus coeruleus would possibly at least partly explain this observation (Jonsson et al. 1974)

Coupled with the decreased ^{3}H NA uptake in cortical and hypothalamic synaptosomes there was also a decreased ^{35}S content (down to 65–70% of control of these subcellular par- ticles) Such concurrent decreases of both the tritium and sulphur label indicate that part of the ^{35}S component is localized to adrenergic vesicles and might be involved in the bind- ing and storage of catecholamines within synaptosomes. In the pons-medulla region, how- ever the increased ^{3}H NA uptake was not associated with increased ^{35}S-content: no change between control and denervated animals was observed. Since this region of the brain has a high myelin content and about 60% of the incorporated sulphur was found associated with the lipid fraction of the synaptosomes (Table II), a change in ^{35}S labelled SMPS might have escaped attention as total ^{35}S was measured. Also the possibility should be kept in mind that the absence of increase of SMPS might partly be related to the formation of new terminals with relatively few and/or immature granules with a low content of SMPS Observations from the density gradient experiments showed that much of the sulphur cor- responded very closely to the main synaptosome distribution as depicted by the tritium curve. The pons was not used in this part of the study because the high lipid content would too strongly interfere with the ^{35}S-labelled SMPS distribution. Of the three regions examined on density gradients the hypothalamus showed a heavier type of synaptosomes equilibrat- ing at 1 20 M sucrose, whilst those of the cerebral cortex and cerebellum were less dense to equilibrated at about 1 10 M sucrose (Pycock and Jonsson 1974). A smaller additional peak of radioactive sulphur concomitant with tritium was observed in the distribution pattern of the hypothalamus. This secondary peak could possibly represent small catecholamine storage vesicles (equilibrating at around 0 75–0 60 M sucrose) released during the prepara- tion and resuspension of the synaptosomes before density gradient centrifugation. Indeed H NA labelled synaptosomes subjected to mild lysis procedure in hypotonic sucrose before gradient centrifugation produce a broad storage vesicle peak in the range 0.75–0.55 M sucrose (Pycock, unpublished observations).

The 20% loss of radioactive sulphur following complete lysis of the crude synaptosomal fraction with distilled water indicates the presence of a soluble releasable component of the sulphur-containing compounds as shown for the acetylcholine stimulated perfused adrenal gland of the cat (Margolis, Jaanus and Margolis 1973) The possible association of this ^{35}S- containing material with catecholamine storage remains to be shown. In addition, there may well be a small fraction of insoluble SMPS associated with the membrane fragments in suspension. Experiments with H NA labelled synaptosomes show that 80–90% of radio- activity is releasable by lysis in distilled water

The observed parallel decrease of H NA uptake into synaptosomes and their ^{35}S con- tent after 6-OH DA treatment and the concomitant distribution of H and ^{35}S on gradients prompted the question about the chemical identity of the sulphur labelled compounds resp. compounds. Indeed, several groups of ^{35}S labelled compounds were verified in the synaptosome fractions from the cortex, hypothalamus and cerebellum the ^{35}S label was almost evenly distributed between sulphatides, sulphated glycoproteins and SMPSs. The three groups of compounds have been in recent years repeatedly identified in preparatio

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The Interstitial Space of Adipose Tissue as Determined by Single Injection and Equilibration Techniques

By

Birgitta Linde and Guy Chisolm*

Received 18 April 1975

Abstract

Linde, B. and G. Chisolm. *The interstitial space of adipose tissue as determined by single injection and equilibration techniques.* Acta physiol. scand. 1975. 95. 383–390.

The interstitial ^{14}C-sucrose space was determined in the subcutaneous adipose tissue of the dog using both single injection-indicator dilution technique and an equilibration-tissue sampling method. ^{131}I-albumin and ^{51}Cr-labelled erythrocytes served as intravascular indicators. The conventional extrapolation method for single injection curves yielded space values several times lower than those measured by the equilibration technique, although sampling was continued until the venous outflow samples contained less than 1 % of peak ^{14}C-sucrose activity. This discrepancy may be due to long transit times, for which the conventional extrapolation procedure does not account. An extrapolation procedure is proposed based on the assumption that the area under the normalized extra- and intravascular curves should be equal. By this procedure as well as by the equilibration technique the interstitial ^{14}C-sucrose space in adipose tissue measures approximately 10 ml · 100 g^{-1}. The single injection technique does not appear applicable for space determinations in adipose tissue during sympathetic nerve stimulation (3–7 Hz), possibly due to "trapping" of the tracers within the tissue.

The single injection—indicator dilution technique (Meier and Zierler 1954) was used to measure the size of the interstitial space in the subcutaneous adipose tissue of the dog. Earlier studies (Öberg and Rosell 1967) have suggested that a decrease in the size of this compartment may occur during sympathetic nerve stimulation. However, the application of the single injection technique to determine space in adipose tissue involved methodological problems. Therefore, a somewhat more detailed study of this method as a measure of extravascular space in adipose tissue was carried out. Spaces obtained by the single injection technique were compared to those measured by an equilibration—tissue sampling method.

Methods

15 female mongrel dogs, fasted overnight were anesthetized with sodium pentobarbital, 30 mg/kg i.v. supplemented as necessary. All animals were tracheotomized and artificial positive pressure breathing was

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SACHS, CH. and G JONSSON, Degeneration of central noradrenaline neurons after 6-hydroxyd newborn animals. *Res Commun. Chem. Path. Pharmac* 1972. 4 203–220.

SINGH, B. and J DE CHAMPLAIN, Altered ontogenesis of central noradrenergic neurons following treatment with 6-hydroxydopamine. *Brain. Res.* 1972. 48. 432–437

SVEJCAR, J and W VAN ROBERTSON, Micro separation and determination of mammalian ac aminoglycans (mucopolysaccharides). *Analyt Biochem.* 1967 18 333–350.

UVNÄS, B., An attempt to explain nervous transmitter release as due to nerve impulse-induced change. *Acta physiol scand.* 1973 87 168–173

VOS, J K. KURIYAMA and E. ROBERTS, Distribution of acid mucopolysaccharides in subcellul of mouse brain. *Brain Res.* 1969 12. 172 179

WESSLER, E., Analytical and preparative separation of acidic glycosaminoglycans by electr barium acetate. *Analyt Biochem.* 1968. 26 439–444

WHITTAKER, U P The synaptosome. In *Handbook of Neurochemistry* Vol. 2. Ed. A. Lajt Press, New York 1969

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gl en when necessary to maintain normal blood gas values. A subcutaneous adipose tissue prepara the inguinal region according to Rosell (1966) was used. The tissue was totally freed from surro tissues, including the skin, except for an artery and vein. The mixed nerve to the tissue as sectioned. flow was measured using a silicone-filled drop counter inserted into the artery. Heparin in doses of .X kg was used as an anticoagulant. Blood flow and blood pressure were recorded continuously on a polygraph. The temperature of the preparation was kept at 37 38°C with the aid of heating lamp tissue was kept moist by covering with a plastic sheet and saline soaked gauze pads.

A equilibration-tissue sampling procedure was used to determine the sucrose space in nine expt and Phelps 1966). The tissue was artificially perfused from a reservoir according to the technique of and Rosell (1962). 100–250 / Cl ^{14}C-sucrose (The Radiochemical Centre, Amersham) and appr. 50 μCl ^{51}Cr labelled red cells were mixed with about 200–300 ml of the dog's blood in the perfusion re which was maintained at 38°C. Thus, an in situ perfusion was established with the effluent venous being discarded. Labelling of the red blood cells was performed according to Owen (1959) usi ^{51}CrO (Atomenergi, Studs ik). The tissue was perfused for approximately 90 min (range 50-110 min average blood flow of 6.6 ml min 100 g^{-1} and at an average pressure of 140 mm Hg at the inflow site. Arterial and enous samples were collected every 10 to 15 min for hematocrit determ and liquid scintillation counting. No statistically significant difference was found in the ^{51}Cr ^{14} between arterial and venous blood after 90 min of perfusion. At the end of the perfusion period the and vein were simultaneously clamped and cut and the tissue was immediately transferred into nitrogen. From the frozen fat tissue, pieces of 1 5 g were cut after removing a thin layer from the The fat pieces were dissolved in Soluene® 350 (Packard Instrument) overnight at 60°C, after which of the liquid were prepared for liquid scintillation counting (Packard Tri Carb 3375). The samples fro experiment were run together with quenched standards. The analytical errors, determined as the co of variation for a single determination, were 0.8 for ^{14}C and 4 6 for ^{51}Cr

Using the assumption that sucrose had totally equilibrated with the tissue, and that the sucrose con concentration is the same in plasma and in interstitial fluid, the total sucrose space (properly expr ml equivalent plasma 100 g^{-} but for simplicity written as ml 100 g^{-}) was calculated as follow

$$\text{Total sucrose space (ml } 100\ g^{-1}) = \frac{\text{cpm } ^{14}C}{\text{cpm } ^{14}C}\ \frac{100\ g\ \text{tissue}}{\text{ml plasma}}$$

Calculation of plasma volume was performed according to the following:

$$\text{Plasma volume (ml } 100\ g^{-}) = \frac{\text{cpm } ^{51}Cr}{\text{cpm } ^{51}Cr}\ \frac{100\ g\ \text{tissue}}{\text{ml blood}}\ \frac{1\ (1\ Hct)}{\text{ml blood}}$$

assuming the venous hematocrit to be equal to the tissue hematocrit. Interstitial sucrose space sucrose space—plasma olume.

The single injection-indicator dilution technique (Meier and Zierler 1954) using ^{131}I-albumin as th vascular indicator was used in six expts. performed during autoperfusion of the tissue. ^{14}C-suc ^{131}I-albumin (1 5 μCi of each pproximately 75 μl) were injected over one s through rubber tu serted into the arterial inflow ca nula. 30 to 50 venous samples were collected dropwise whe ul 5.5 to 35 min after the jection.

In order to avoid recirculation of radioactive blood during the sampling period, the enous bl not returned to the animal until after the end of each sampling period. In high blood flow expts. bl taken from the nimal before the njection and returned during the sampling period t keep the pressure constant. This was less of a problem since relatively large dogs were used (mean b. 17 make the sampling times shorter prostaglandin E_1 was administered in 2 expts. t increase the blo rate.

Stimulation of the sympathetic nerv supply (12 V 2 ms, 3–7 H) was performed via bipolar ele trodes w th square wa e impulses from Grass stimulator. Injections were performed approximat min after the beginning of stimulation

Assumi g that mixing of blood and ndicator was complet in the arterial ca ula, total sucros and plasma olume were calculated from the curves describing enous concentration ersus time, ac to $V = Q_{pl}\ \bar{t}$ (Meier and Zierler 1954), (where V is the volume of the system in ml equivalent pl 100 g Q_{pl} is the plasma flow rate ml min 100 g nd $\bar{t}$ the mean transit time in min for t cator through the system). The interstitial sucrose pace was calculated as the difference between t sucrose space and the plasma olume (PV) Corrections were made for the olume of the sampling c which measured approximately 0.5 ml (Zierler 1962).

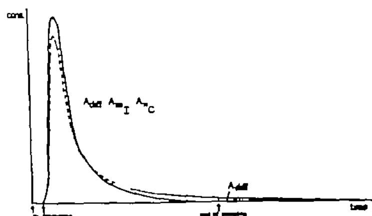


Fig. 1. Schematic picture of venous concentration-time curves for ^{131}I-albumin (the intravascular indicator —) and ^{14}C-sucrose (the extravascularly diffusing tracer, —) following a single arterial injection. The venous concentrations have been normalized with respect to those of the fluid injected. The "tail" of the partially-recovered ^{14}C curve is obtained by monoexponentially extrapolating (. .) to the base line, in this case from the last sample (and in other cases from the first sample which contains no ^{131}I activity), in such a way that the area under the extrapolated part of the curve (A_{diff}) equals the difference in area between the totally-recovered ^{131}I curve (A_{m_I}) and the recovered part of the ^{14}C curve (A_{m_C}).

In the single injection experiments sampling was performed in each study until the ^{131}I activity could no longer be distinguished from the background level. The ^{14}C activity however was somewhat (0.09–0.65% of the peak activity) above the background level at the end of the sampling period. Therefore extrapolation of the ^{14}C curve had to be used in order to obtain the mean transit time. Extrapolation was performed in two ways, firstly by the conventional method of Hamilton *et al.* (1932), using the time intervals just before ending the sampling as a basis for the extrapolation. Secondly extrapolation was performed according to the method shown in Fig. 1 which is based on the assumption that ^{14}C should recover in the venous out-

Table 1. Total and interstitial ^{14}C-sucrose space determined by an equilibration technique in dog subcutaneous adipose tissue. Blood flow, mean arterial blood pressure, venous hematocrit, sucrose spaces and plasma volume are given, the two latter as means ± S.E. for each expt. together with mean ± S.E. for the whole material.

Exp.	Determinations/fat pad	Blood flow ml min 100 g^{-1}	Mean arterial blood pressure mm Hg	Venous Hct	Plasma volume ml 100 g^{-1}	Total sucrose space ml 100 g^{-1}	Interstitial sucrose space ml 100 g^{-1}
[illegible]	2	5.4	175	32	8.2 ± 0.7	17.8 ± 1.8	9.6 ± 1.2
[illegible]	7	9.9	120	47	5.0 ± 1.2	12.1 ± 1.7	7.1 ± 1.0
[illegible]	2	3.8	100	28	9.7 ± 0.1	20.0 ± 2.0	10.3 ± 2.0
[illegible]	5	4.0	150	37	4.2 ± 0.2	—	—
[illegible]	6	9.0	155	40.5	4.4 ± 1.2	15.1 ± 2.3	10.7 ± 1.5
[illegible]	7	1.2	175	31	—b	23.5 ± 2.1	—b
[illegible]	5	8.9	170	49.5	5.2 ± 0.8	4.0 ± 2.7	18.8 ± 2.2
[illegible]	8	10.0	100	40	7.9 ± 1.9	18.2 ± 2.1	10.3 ± 1.2
[illegible]	4	3.9	140	40	8.3 ± 3.5	15.7 ± 1.9	7.4 ± 1.8
				Whole material	6.6 ± 0.8 (n = 8)	18.3 ± 1.5 (n = 8)	10.6 ± 1.5 (n = 7)

a Equilibration not complete.
b Too little ^{51}Cr in the blood.

TABLE II. Total and interstitial ^{14}C sucrose space determined by the single injection technique in dog skeletal muscle ... in Fig. 1 time of sampling, time used for start of extrapolation, venous hematocrit, plasma flow ... extravascular tracer are given for each individual injection as well as mean ± S.E. in the ...

Exp	Inj	Time of sampling min	Time used for start of extrapol. min	Venous Hct	Plasma flow ml min^{-1} 100 g^{-1}	Plasma volume ml 100 ...
1	1	5.5	5.5	46	9.3	40
	2	5.5	5.5	45	9.1	44
	3	5.5	5.5	45.5	8.9	41
2	1	5.5	5.5	8.5	17.6	71
	2	5.5	5.5	28	16.8	83
3	1	5.5	5.5	40	17.5	71
	2	5.5	5.5	39	17 7	75
4	1	5.5	5.5	48	8.9	34
	2	5.5	5.5	44	9.0	31
5	1	20	13 20	42	3.4	64
		20	13 '0	41	4.8	42
6	1	35	10 35	43	6.1	77
	2	35	18 35	42	3.8	71
			Whole material $\bar{x}$ (n = 6)	S.E.	10.5 2.3	62 09

Value at end of ^{131}I curve.

flow to the same extent as ^{131}I Thus, the area of the ^{131}I curve is taken to represent 100 recovery for the extravascular indicator Careful registration of the background activity as well as of the concentrations in the fluid injected is essential n this connection. Several blanks were taken bet een injections. The concentrations in the injected fluid were determined as an verage of 10 samples. The error in the determination of the ratio between ^{14}C and ^{131}I i the injected fluid expressed as the coefficient of ariation for single determinatron was 1 7 .

The ^{14}C curve was monoexponentially extrapolated from the first sample containing no trace of or from a later sample, in such a way that the rea under the extrapolated part of the curve equals the difference (A_{4m}) between the totally-recovered ^{131}I curve (A_{131}) and the only partially-recovered ^{14}C curve (A_{14C}). In this way it is possible to define the whole ^{14}C curve and, thus, its point of gravity can be determined, giving the $\bar{t}$ for ^{14}C-sucrose.

Standard statistical procedures were used, the paired t-test being employed when applicable. Data are presented as means ± S.E.

Results

Equilibration technique (Table I)

After approximately 90 min of perfusion the total sucrose space measured 18.3 ± 1.5 ml 100 g^{-} tissue and its extravascular portion 10.6 ± 1 5 ml 100 g^{-} Although the venous hematocrit was somewhat low in three of these expts., no relationship appeared to exist between space values and hematocrit The plasma volume calculated from the tissue and blood content of Cr labelled red cells measured 6.6 ± 0 8 ml 100 g This is likely to be a minimum value, since the tissue hematocrit is probably lower than the venous hematocrit

...ment. Spaces calculated by extrapolation according to Hamilton *et al.* (1932) and by method proposed ... as well as ^{14}C-sucrose activity at last sample and A_{diff}, the difference in area between the intra- and ... the mean value for each expt.

[illegible]	^{14}C activity % of max	A_{diff} of $A_{tot\,I}$	Total sucrose space (Fig. 1) ml 100 g^{-1}	Interstitial sucrose space (Fig. 1) ml 100 g^{-1}
	0.25	3.1	8.1	4.1
	0.24	3.1	9.0	4.6
	0.23	3.4	8.7	4.6
	0.28	3.0	14.8	7.0
	0.22	4.6	19.1	10.6
	0.22	6.4	26.4	18.6
	0.30	5.0	22.4	14.5
	0.17	4.6	10.0	6.6
	0.19	5.7	11.5	8.3
	0.22	6.7	18.0	12.0
	0.08	4.4	18.5	12.5
	0.30	7.1	20.9	14.7
	0.11	4.6	20.4	14.2
	0.61	5.7	22.6	14.9
	0.09	1.4	23.3	15.6
	0.43	4.8	21.2	13.4
	0.34	1.4	20.8	13.0
	0.30^{a}	5.0^{a}	17.1	10.8
	0.07	0.5	2.5	1.9

Single injection technique (Table II)

The plasma volume was 6.2 ± 0.9 ml 100 g^{-1} which is in good agreement with the value obtained from the content of ^{51}Cr labelled red cells in the tissue. The coefficient of variation of a single determination of the plasma volume was 3.4%.

Interstitial space, extrapolation according to Hamilton et al. (1932). After 5–13 min of observation, which was enough for complete washout of the intravascular indicator, the ^{14}C-sucrose activity in the venous outflow was only about 0.3% of the peak activity. Despite this low concentration of the extravascular tracer, the interstitial space, calculated on the basis of Hamilton's method for extrapolation, measured only 2.5 ml 100 g^{-1}. This is several times smaller than the corresponding value obtained by the equilibration technique. When the time of observation was extended (expts. 5 and 6), larger space values were obtained by this extrapolation method.

Interstitial space, extrapolation according to Fig. 1. Using this method of extrapolation, from the first sample where the ^{131}I-albumin activity was zero, the interstitial sucrose space measured 10.8 ± 1.9 ml 100 g^{-1} and the total sucrose space 17.1 ± 2.5 ml 100 g^{-1}. These values are similar to those obtained by the equilibration technique. For the 4 injections in expts. 5 and 6, extrapolation was also carried out from a later point of observation. Only minor differences in the space values were observed when extrapolating from the first sample which showed no trace of ^{131}I-albumin activity compared to later samples. The reproducibility

TABLE II Total and interstitial ^{14}C-sucrose space determined by the single injection technique in dog sub... in Fig. 1 time of sampling, time used for start of extrapolation, venous hematocrit, plasm... extravascular tracer are given for each individual injection as well as mean ± S.E. in th...

Exp	Inj	Time of sampling min	Time used for start of extrapol. min	Venous Hct	Plasma flow ml min^{-1} 100 g^{-1}	P... v... m...
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	2	5.5	5.5	28	16.8	8
3	1	5.5	5.5	40	17.5	7
	2	5.5	5.5	39	17.7	1
4	1	5.5	5.5	48	8.9	3
	2	5.5	5.5	44	9.0	3
5	1	20	13 20	42	5.4	6
	2	20	13 20	41	4.8	6
6	1	35	10 35	43	6.1	7
	2	35	18 35	42	3.8	7
			Whole material $\bar{x}$		10.5	6
			(n = 6)	S.E.	2.3	8

Value at end of ^{131}I curve.

flow to the same extent as ^{131}I. Thus, the area of the ^{131}I curve is taken to represent 100 % recovery ... extravascular indicator. Careful registration of the background activity as well as of the concentrat... the fluid injected is essential in this connection. Several blanks were taken between injections. Th... centrations in the injected fluid were determined as an average of 10 samples. The error in the dete... tion of the ratio between ^{14}C and ^{131}I in the injected fluid expressed as the coefficient of variation ... single determination was 1.7 %.

The ^{14}C curve was monoexponentially extrapolated from the first sample containing no trace ... or from a later sample, in such a way that the area under the extrapolated part of the curve equals ... ference (A_{diff}) between the totally recovered ^{131}I curve (A_{131I}) and the only partially-recovered ^{14}C (A_{14C}). In this way it is possible to define the whole ^{14}C curve and thus, its point of gravity can be ... mined, giving the $\bar{t}$ for ^{14}C-sucrose.

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Equilibration technique (Table I)

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...quences, based on studies in skeletal muscle, was given by Lassen and Sejrsen (1971), ...o stated that "monoexponential extrapolation as made before the appearance of the final ...onential part of the outflow curve, can cause considerable error in determination of the ...n transit time and hence of the volumes of distribution".

...fhus, in certain organs and tissues, e.g. in heart and skeletal muscle, but not in liver, a ...stantial part of the extravascular space has long transit times. When these long transits ...not properly accounted for, considerable errors in the volumes of distribution may result. ...suggest that the discrepancy found in adipose tissue between values of the interstitial ...re obtained by conventional extrapolation of single injection curves and those deter...ned by the equilibration method is caused by such long transits for which the Hamilton ...thod of extrapolation does not account. This explanation appeared even more likely ...ce, after extending the time of observation of the venous outflow, the space values ob...ned by the conventional extrapolation method showed an increase, compared to the ...ues calculated by extrapolation from an earlier point of registration.

Theoretically, the most correct way of accounting for the long transit times is certainly ...e method deduced by Lassen and Sejrsen (1971), where the monoexponential tail is found ...simultaneous outflow and residue detection. We propose another procedure for consider...g the long transits, based only on outflow detection. The method requires registration of ...e entire curve of the intravascular tracer, the area of which is assumed to represent 100% ...covery for the extravascular indicator. This assumption appears valid if the indicators are ...ert and if changes in blood flow do not distort the curves to a different extent. However, ...obtain the most accurate measurements possible, utmost care must be taken in the deter...ination of the areas, by frequent sampling, by careful registration of the background as well ...s by accurate determination of the concentrations in the fluid injected. When these pre...utions were taken in the present study, the coefficient of variation for a single determina...on of the interstitial space was 19%. No marked difference in the space values was seen ...hether extrapolation was performed early or late after the point where the ^{131}I-albumin ...tivity was zero.

Attempts to apply the single injection technique during sympathetic nerve stimulation ...(1-7 Hz) resulted in unreasonably high values of the interstitial space, in some cases even ...alues above the tissue volume. This may be due partly to the systematic change of flow ...aking place in adipose tissue during nerve stimulation, since attempts to correct for this ...low factor resulted in lower values. However, during nerve stimulation other factors may ...lso influence the rate of outflow of the indicators. We suggest that "trapping", which has ...been described for the outflow of FFA from adipose tissue (Rosell 1966), may contribute to ...he high values, since obvious overestimations occurred for both intra- and extravascular ...spaces.

In summary, the present study demonstrates the importance of considering the longest ...transit times when calculating interstitial space from single injection curves in subcutaneous ...adipose tissue. An extrapolation procedure is proposed, based on outflow detection of an ...intra- and extravascular indicator. It requires registration of the entire outflow curve for the ...intravascular tracer, the area of which is thought to represent 100% recovery of the extra...vascular indicator. Using this procedure good agreement is found between space values

for the determination of the interstitial space, expressed as the coefficient of variation single determination, was 19 1 %.

Sympathetic nerve stimulation

Attempts were also made to apply the single injection technique during sympathetic stimulation (3–7 Hz). Using the extrapolation procedure according to Fig. 1 quite unrea values for the intra and extravascular spaces were obtained. Interstitial space values gr than the tissue volume were calculated in some cases. Since the capacitance has been sh to decrease during nerve stimulation (Öberg and Rosell 1967) the high intravascular vol measured are probably due to an error in the measuring technique. During stimulatio sympathetic nerves to adipose tissue, blood flow decreases initially After some mi vasoconstriction starts to diminish, resulting in a gradual increase in blood flow This tinuous flow change may partly be responsible for the high values. Attempts to corre the flow alteration, by integrating the product of venous concentration and its correspo flow values over time, resulted in more reasonable space values in 2 injections. How in the other injections, no obvious explanation for the high intra- and extravascular s values could be found Therefore no further attempts were made to apply the single inje technique during nerve stimulation conditions.

Discussion

The aim of the present study was initially to measure the size of the interstitial spac subcutaneous adipose tissue and to investigate whether a decrease of this space takes pl during sympathetic nerve stimulation, as indicated by earlier studies (Öberg and Rosell 19 The single injection technique (Meier and Zierler 1954), which enables repeated meas ments to be made in the same tissue, was chosen in preference to equilibration meth However in spite of sampling until less than one per cent of the peak ^{14}C-sucrose act remained in the venous outflow the space values obtained by this technique, using the c ventional extrapolation method of Hamilton *et al.* (1932), were several times smaller th previously reported from adipose tissue of other species and locations using equilibrat techniques in vitro (Denton *et al* 1966, Björntorp *et al* 1966, Englhardt *et al* 1971). T were also much smaller than values of adipose tissue water obtained by Fredholm a Fronek (1974). Therefore the interstitial space in this tissue was determined using an eq bration—tissue sampling method (Law and Phelps 1966). The duration of perfusion w chosen to give practically complete equilibration between tissue and blood. The sp values thus obtained were considerably larger than those calculated from the single injectio experiments, using the conventional extrapolation method, but in good agreement with t previous estimates from other studies mentioned above.

A discrepancy between extravascular spaces obtained by the conventional extrapolati of single injection curves and those determined by equilibration methods has also be observed in heart muscle, in spite of recoveries near 100% (Ziegler and Goresky 1971). I liver the space values based on extrapolation according to Hamilton *et al* agreed well wi other estimates of the extravascular space (Goresky 1963). A likely explanation for su

Acta physiol. scand. 1975, 95, 391–399

From the Department of Physiology, University of Kuopio, Finland

Comparison of the Effects of Physical Exercise, Cold Acclimation and Repeated Injections of Isoprenaline on Rat Muscle Enzymes

By

M. N. E. Harri and J. Valtola

Received 5 May 1975

Abstract

Harri, M. N. E. and J. Valtola. *Comparison of the effects of physical exercise, cold acclimation and repeated injections of isoprenaline on rat muscle enzymes.* Acta physiol. scand. 1975, 95, 391–399.

The metabolic effects on rat cardiac and skeletal muscle of a strenuous program of swimming, of cold acclimation and of isoprenaline treatment (0.3 mg/kg daily for 5 five-day weeks) were compared. Exercised and cold-exposed rats gained less body weight than did controls or isoprenaline-treated rats. In all treated groups the heart and the interscapular brown adipose tissue hypertrophied. The size of the adrenals increased only in isoprenaline-treated animals. Cold-acclimation and physical training increased and isoprenaline treatment reduced or did not affect the activities of succinate dehydrogenase, malate dehydrogenase and citrate synthase of cardiac muscle. In skeletal muscle all treatments resulted in increased activities of these enzymes. Of the anaerobic enzymes analysed, only the activity of hexokinase increased in response to the treatments used. This increase was the same in cardiac as in skeletal muscle, but it was significantly greater with isoprenaline-treatment than with training or with cold-acclimation. The activities of lactate dehydrogenase and phosphofructokinase did not differ significantly. All treatments improved cold tolerance, but only swimming exercise and cold acclimation significantly increased tolerance to exercise. It is concluded that prolonged stimulation of adrenergic β-receptors by catecholamines is responsible for the metabolic changes observed.

Key words: Gastrocnemius muscle, cardiac muscle, enzyme activity, physical exercise, cold acclimation, isoprenaline treatment, cold tolerance, exercise tolerance

It is well known that skeletal and cardiac muscle adapt to physical exercise by increasing the number and size of mitochondria and content of mitochondrial enzymes (Holloszy 1973). On the other hand, attempts to demonstrate changes in the activities of enzymes involved in glycolysis have led to contradictory results (Holloszy *et al.* 1971, Gollnick and Hermansen 1973). The changes in muscle enzymes as a result of physical training are very similar to those achieved by cold-acclimation (Hannon 1963, Hamilton and Ferguson 1972). Furthermore, acclimation to cold increases the amount of time a rat can swim before exhaustion (Bazhabova 1960, Dawson *et al.* 1970). A relationship between physical fitness and the ability to tolerate cold has been demonstrated in humans (Adams and Heberling 1958, Heberling and Adams 1961, Keatinge 1961, Lange Andersen 1966) and in rats (Stromme and Hammel 1967). On the other hand, repeated injections of isoprenaline results in improved

measured by the single injection technique and those determined by an equilibration method. Both methods give values for the interstitial space in adipose tissue of approximately 10 ml 100 g^{-1} or about half of the body average. Application of the single injection technique to the determination of interstitial space during sympathetic nerve stimulation has not been successful in the present study.

This study was supported by grants from the Swedish Medical Research Council (B75-40X 3511-04) and by the Karolinska Institutet, Stockholm.

References

Björntorp, P., B. Hood and A. Martinsson, The sucrose space of human subcutaneous adipose tissue in obesity. *Acta med. scand.* 1966, *180*, 123–127.

Denton, R. M., R. E. Yorke, and P. J. Randle, Measurement of concentrations of metabolites in adipose tissue and effects of insulin, alloxandiabetes and adrenaline. *Biochem. J.* 1966, *100*, 407–419.

Engelhardt, A., H. Liebermeister, Th. Reuter and K. Irmscher, Analyse der Wasserräume und des Lipidgehalts des menschlichen subcutanen Fettgewebes. *Z. klin. Chem. Biochem.* 1971, ... 356–368.

Fredholm, B. B. and A. Fronek, High energy phosphate compounds in adipose tissue: The effect of hemorrhage. *Acta physiol. scand.* 1974, *91*, 165–171.

Goresky, C. A., A linear method for determining liver sinusoidal and extravascular volumes. *Amer. J. Physiol.* 1963, *204*, 626–640.

Hamilton, W. F., J. W. Moore, J. M. Kinsman and J. M. Spurling, Studies on the circulation. IV. Further analysis of the injection method, and of changes in hemodynamics under physiological and pathological conditions. *Amer. J. Physiol.* 1932, *99*, 534–551.

Lassen, N. A. and P. Sejrsen, Monoexponential extrapolation of tracer clearance curves in kinetic analysis. *Circulat. Res.* 1971, *29*, 76–87.

Law, R. O. and C. F. Phelps, The size of the sucrose, raffinose, and inulin spaces in the gastrocnemius muscle of the rat. *J. Physiol.* (Lond.) 1966, *186*, 547–557.

Meier, P. and K. L. Zierler, On the theory of the indicator-dilution method for measurement of blood flow and volume. *J. appl. Physiol.* 1954, *6*, 731–744.

Öberg, B. and S. Rosell, Sympathetic control of consecutive vascular sections in canine subcutaneous adipose tissue. *Acta physiol. scand.* 1967, *71*, 47–56.

Owen, C. A., *Diagnostic radioisotopes.* Charles C. Thomas, Springfield, USA 1959, p. 165.

Renkin, E. M. and S. Rosell, The influence of sympathetic adrenergic vasoconstrictor nerves on transport of diffusible solutes from blood to tissues in skeletal muscle. *Acta physiol. scand.* 1962, *54*, 223–...

Rosell, S., Release of free fatty acids from subcutaneous adipose tissue in dogs following sympathetic nerve stimulation. *Acta physiol. scand.* 1966, *67*, 343–351.

Ziegler, W. H. and C. A. Goresky, Transcapillary exchange in the working left ventricle of the dog. *Circulat. Res.* 1971, *29*, 181–207.

Zierler, K. L., Theory of use of indicators to measure blood flow and extracellular volume and calculation of transcapillary movement of tracer. *Circulat. Res.* 1963, *12*, 464–471.

Zierler, K. L., Theoretical basis of indicator-dilution methods for measuring flow and volume. *Circulat. Res.* 1962, *10*, 393–407.

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References

BJÖRNTORP P B. HOOD and A. MARTINSSON, The sucrose space of human subcutaneous adipose tissue in obesity *Acta med. scand.* 1966, *180*. 123–127

DENTON, R. M., R. E. YORKE, and P J RANDLE, Measurement of concentrations of metabolites in adipose tissue and effects of insulin, alloxandiabetes and adrenaline. *Biochem. J* 1966. *100*. 407–419.

ENGLHARDT A., H. LIEBERMEISTER, Th. REUTER and K. IRMSCHER, Analyse der Wasserräume und Lipidgehalts des menschlichen subcutanen Fettgewebes. *Z klin. Chem. Biochem.* 1971. *9* 356–364.

FREDHOLM, B. B. and A. FRONEK, High energy phosphate compounds in adipose tissue: The effect of hemorrhage. *Acta physiol. scand.* 1974. *91* 165–171

GORESKY C. A., A linear method for determining liver sinusoidal and extravascular volumes. *Amer J Physiol.* 1963. *204* 626–640.

HAMILTON, W F J W MOORE, J M. KINSMAN and J M. SPURLING, Studies on the circulation. IV Further analysis of the injection method, and of changes in hemodynamics under physiological and pathological conditions. *Amer J Physiol.* 1932. *99* 534–551

LASSEN, N. A. and P SEJRSEN, Monoexponential extrapolation of tracer clearance curves in kinetic analysis. *Circulat Res.* 1971 *29* 76–87

LAW R. O and C. F PHELPS, The size of the sucrose, raffinose, and inulin spaces in the gastrocnemius muscle of the rat. *J Physiol.* (Lond.) 1966. *186*. 547 557

MEIER, P and K. L. ZIERLER, On the theory of the indicator-dilution method for measurement of blood flow and volume. *J appl. Physiol.* 1954. *6*. 731–744.

ÖBERG, B. and S. ROSELL, Sympathetic control of consecutive vascular sections in canine subcutaneous adipose tissue. *Acta physiol. scand.* 1967 *71* 47 56.

OWEN, C. A., *Diagnostic radioisotopes*. Charles C. Thomas, Springfield, USA 1959. p. 165.

RENKIN, E. M. and S. ROSELL, The influence of sympathetic adrenergic vasoconstrictor nerves on transport of diffusible solutes from blood to tissues in skeletal muscle. *Acta physiol. scand.* 1962. *54*. 223–...

ROSELL, S., Release of free fatty acids from subcutaneous adipose tissue in dogs following sympathetic nerve stimulation. *Acta physiol. scand* 1966. *67* 343–351

ZIEGLER, W H. and C. A. GORESKY Transcapillary exchange in the working left ventricle of the dog. *Circulat Res.* 1971 *24* 181 207

ZIERLER, K. L., Theory of use of indicators to measure blood flow and extracellular volume and calculation of transcapillary movement of tracer *Circulat Res.* 1963 *12* 464–471

ZIERLER, K L., Theoretical basis of indicator-dilution methods for measuring flow and volume. *Circulat Res.* 1962. *10*. 393–407

TABLE I. Body weight and weight of hearts, interscapular brown adipose tissues (ISBAT) and adrenals in control, cold-acclimated, trained and isoprenaline (ISO)-treated rats.

Weight	Control	Cold-acclimated	Trained	ISO-treated
Body, g	303 ± 9.4[a] (9)[b]	276 ± 9.9[e] (9)	275 ± 10.9[c] (7)	302 ± 9.3 (6)
Heart, mg	822 ± 13.3	956 ± 21.9[e]	911 ± 13.1	1 233 ± 22.5[e]
ISBAT, mg	383 ± 11.5	823 ± 34.6	752 ± 58.0[e]	513 ± 16.7[e]
Adrenals, mg	67.7 ± 2.4	69.7 ± 1.7	68.6 ± 1.5	76.5 ± 1.5[d]

[a] Mean ± S.E.
[b] Number of rats.
Significant difference from the controls:
[c] p<0.05, [d] p<0.01 and [e] p 0.001 (Students' t-test).

...trol of all (p 0.001 Student's t-test). All the treatments used resulted in hypertophy of the ISBAT tissue as is seen by its increased weight after the treatments. In the isoprenaline-treated rats the increase was smaller than that in the cold-acclimated (p 0.001) or trained rats (p 0.005). Only repeated injections of isoprenaline increased the weight of the adrenals.

...uscle enzymes

Cardiac muscle. The results in Table II show that both the cold-acclimation and the swimming ...cise used increased *the activities of the aerobic enzymes of the myocardium. Mean* SDH activity increased 41% with cold-acclimation and 40% with training. The activity of CS was increased 15% and 19% and that of MDH 23% and 20% in cold-acclimated and trained rats, respectively. By contrast, in isoprenaline-treated rats, the activity of CS was decreased by 29%. The decreases in the mean activities of MDH (−6%) or SDH (−14%) were not statistically significant. Of the anaerobic enzymes only HK showed significant changes in response to the treatments used. The HK activity of heart homogenate of the isoprenaline-treated rats was 35% higher than that of heart homogenate of the controls. Cold-acclimation and exercise in turn caused a 15% and a 10% increase over the control level, respectively. The increase in HK-activity in the isoprenaline-treated rats was signifi-

TABLE II. Activities of cardiac muscle enzymes and protein content in control, cold-acclimated, trained and isoprenaline (ISO)-treated rats.

Enzyme	Control	Cold-acclimated	Trained	ISO-treated
Succinate dehydrogenase	45.2 ± 3.37	63.5 ± 3.63[d]	63.0 ± 4.44[d]	40.3 ± 3.57
Malate dehydrogenase	540 ± 16.4	662 ± 34.9[c]	624 ± 19.0[d]	525 ± 31.5
Citrate synthase	74.1 ± 1.35	85.0 ± 3.07[e]	88.0 ± 4.95[e]	52.4 ± 3.17[d]
Hexokinase	5.91 ± 0.20	6.85 ± 0.53[a]	6.55 ± 0.29[b]	8.02 ± 0.41
Phosphofructokinase	48.1 ± 2.86	42.8 ± 1.93	49.0 ± 6.40	40.3 ± 6.52
Lactate dehydrogenase	555 ± 28.7	639 ± 30.2	828 ± 131.8	636 ± 55.4
Protein	78.2 ± 2.89	87.0 ± 2.51	84.5 ± 2.62	64.1 ± 1.63[e]

Enzyme activities are expressed as μmoles of substrate utilized per min per g wet weight, and protein content as mg protein in cell free homogenate from 1 g of muscle. Values are means ± S.E. Numbers of rats are identical with those given in Table I.
Significant difference from the control: [a] p<0.05, [b] p<0.02, [c] p 0.01 and [d] p 0.001 (Students' t-test).

cold tolerance of rats, but does not extend swimming time (LeBlanc, Vallieres and Vachon 1972). The present study was undertaken in order to compare the metabolic changes caused by strenuous physical training in the muscle with those caused by cold-acclimation and by repeated injections of isoprenaline.

Material and methods

Animals

Four groups of male albino rats of Wistar/af/Han/Mal/(Han 67) strain weighing approximately ... at the beginning of the experiments were used in these studies. The first group consisted of control animals and the second group of cold acclimated rats (4°C for 5 weeks). The rats of the third group were exercised by swimming for 1/2 h daily in water at 30°C. The swimming program was performed five times per week for 5 weeks. A weight of 5 g was attached at the base of the tail, and the weight was gradually increased during the first week to the final weight of 10 g. The rats of the fourth group received daily subcutaneous injections of isoprenaline (0.3 mg/kg) prepared fresh each day in an olive oil suspension. The injections were given five times per week for 5 weeks. The rats were killed by decapitation and hearts, adrenals and interscapular brown adipose tissues (ISBAT) were removed and weighed. The weights were adjusted to a common body weight by analysis of covariance.

Enzyme assays

Samples of the gastrocnemius muscle and of the left ventricular muscle were immediately frozen in liquid nitrogen. The samples were stored frozen at −80°C until assayed. The muscle samples were then homogenized in a Potter Elvehjem glass homogenizer in Tris-HCl buffer (0.1 m, pH 7.6) to a 2 ... homogenate and centrifuged for 10 min at 1 000 g at 4 C to remove unbroken cells and particulate debris. The supernatants were used for determination of the enzyme activities. Measurements were made at ... with a Cary 118 spectrophotometer equipped with a recorder.

Succinate dehydrogenase (SDH) activity was analysed according to Earl and Korner (1965), malate dehydrogenase (MDH) according to Ochoa (1955) and citrate synthase (CS) activity according to S... (1962). The activities of hexokinase (HK), phosphofructokinase (PFK) were determined according to ... *et al.* (1969) and Boström *et al.* (1973), respectively. For the determination of lactate dehydrogenase (LDH) activity the standardized method of Biochemical Boehringer was used. All enzyme activities are expressed as μmoles substrate utilized per min per g wet tissue weight. The protein contents of homogenates were estimated by the phenol method (Lowry *et al.* 1951).

Cold tolerance and swimming time test

Cold tolerance and swimming time tests were made after the fourth week of treatment. The same animal was never used for both of the tests.

Cold tolerance was measured at ... 18 C. Four animals in a cage, each belonging to a different group, were placed at that temperature. Colonic temperatures were obtained with a thermocouple inserted to a depth of 4 cm.

The swimming time was measured in water at 30°C with a load of 5 ... of the body weight attached at the base of the tails. Animals in all four groups were used simultaneously in the test. The end point for swimming time was calculated when the rats remained more than 15 s under the water (LeBlanc et al. 1972).

Results

Body and organ weights

At the beginning of the experiments the body weights of the rats were identical in all experimental groups. At the end of the 5-week experimental period the control and isoprenaline-treated animals weighed significantly more than the cold-acclimated or exercised animals. Thus the cold-acclimated and the exercised rats gained less weight than did rats of the other groups (Table 1). However, the hearts of the control rats were lighter than those of the other groups, while the hearts of the isoprenaline-treated rats were

TABLE I. Body weight and weight of hearts, interscapular brown adipose tissues (ISBAT) and adrenals in control, cold-acclimated, trained and isoprenaline (ISO)-treated rats.

Weight	Control	Cold-acclimated	Trained	ISO-treated
Body, g	305 ± 9.4 (9)[b]	276 ± 9.5[a] (9)	275 ± 10.9[a] (7)	302 ± 9.3 (6)
Heart, mg	822 ± 13.3	956 ± 21.9[c]	911 ± 15.1	1 211 ± 22.5[c]
ISBAT, mg	383 ± 11.5	823 ± 34.6	712 ± 58.0[c]	513 ± 16.7[c]
Adrenals, mg	67.2 ± 2.4	68.7 ± 1.7	68.6 ± 1.9	76.5 ± 1.5

Mean ± S.E.
Number of rats.
Significant difference from the control:
0.05, p 0.01 and p 0.001 (Student t-test).

of all (p 0.001, Student's t-test). All the treatments used resulted in hypertophy of the ISBAT tissue as is seen by its increased weight after the treatments. In the isoprenaline-treated rats the increase was smaller than that in the cold-acclimated (p < 0.001) or trained rats (p 0.005). Only repeated injections of isoprenaline increased the weight of the adrenals.

Muscle enzymes

Cardiac muscle. The results in Table II show that both the cold-acclimation and the swimming exercise used increased the activities of the aerobic enzymes of the myocardium. Mean SDH activity increased 41% with cold-acclimation and 40% with training. The activity of CS was increased 15% and 19% and that of MDH 23% and 20% in cold-acclimated and trained rats, respectively. By contrast, in isoprenaline-treated rats, the activity of CS was decreased by 29%. The decreases in the mean activities of MDH (6%) or SDH (−14%) were not statistically significant. Of the anaerobic enzymes only HK showed significant changes in response to the treatments used. The HK activity of heart homogenate of the isoprenaline-treated rats was 35% higher than that of heart homogenate of the controls. Cold-acclimation and exercise in turn caused a 15% and a 10% increase over the control level, respectively. The increase in HK-activity in the isoprenaline-treated rats was signifi-

TABLE II. Activities of cardiac muscle enzymes and protein content in control, cold-acclimated, trained and isoprenaline (ISO)-treated rats.

Enzyme	Control	Cold-acclimated	Trained	ISO-treated
Succinate dehydrogenase	45.2 ± 3.37	63.5 ± 3.63[d]	63.0 ± 4.24[c]	40.3 ± 3.57
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Phosphofructokinase	49.1 ± 2.86	42.8 ± 1.93	49.0 ± 6.40	40.3 ± 6.52
Lactate dehydrogenase	555 ± 28.7	639 ± 30.2	829 ± 131.8	634 ± 55.4
Protein	78.2 ± 2.89	87.0 ± 2.51	84.5 ± 2.62	64.1 ± 1.68[c]

Enzyme activities are expressed as µmoles of substrate utilized per min per g wet weight, and protein content as mg protein in cell free homogenate from 1 g of muscle. Values are means ± S.E. Numbers of rats are identical with those given in Table I.
Significant difference from the control: p 0.05, p 0.02, p 0.01 and p < 0.001 (Student's t-test).

TABLE III Activities of gastrocnemius muscle enzymes and protein content in control, cold-acclimated, trained and isoprenaline (ISO)-treated rats.

Enzyme	Control	Cold-acclimated	Trained	ISO-treated
Succinate dehydrogenase	6.35±0.32	8.25±0.50[a]	10.15±1.04[d]	9.85±[illegible][d]
Malate dehydrogenase	124±5.94	165±12.3	186±9.4[d]	171 [illegible][d]
Citrate synthase	13.4±0.38	15.8±1.82[b]	20.2±1.24[d]	19.6±[illegible][d]
Hexokinase	1.09±0.04	1.27±0.04	1.26±0.08[a]	1.73±[illegible][d]
Phosphofructokinase	16.1±1.32	15.7±0.92	13.9±0.63	15.5±[illegible]
Lactate dehydrogenase	865±42.3	920±34.0	861±19.3	[illegible]±[illegible]
Protein	68.1±1.82	68.1±2.76	66.6±2.01	67.1±[illegible]

Enzyme activities are expressed as μmoles of substrate utilized per min per g wet weight, and protein con as mg protein cell free homogenate from 1 g of muscle. Values are means ± S.E. Numbers of an identical with those given in Table I.
Significant difference from the control $p < 0.05$, [b] $p < 0.02$, $p < 0.01$ and [d] $p < 0.001$ (Stude t test).

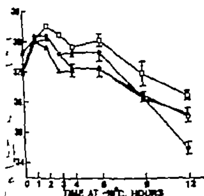
cantly greater than that in the cold-acclimated ($p < 0.05$) or trained rats (p 0.05). protein concentration of the cell-free homogenate of the myocardium was signific over (12%) the control concentration for the cold-acclimated rats. A similar tendency was observable for the trained animals ($p < 0.1$). On the other hand, the heart homog from the isoprenaline-treated animals had a significantly lower protein concentration t the corresponding homogenate from other groups (−17% in comparison with the contr

Gastrocnemius muscle The results in Table III show that all the treatments used ind a rise in the activities of aerobic enzymes of gastrocnemius muscle. Mean activity of S increased 58%, mean activity of CS 50% and mean activity of MDH 50%, respect with exercise. The corresponding increases for SDH, CS and MDH were 30, 35 and with cold-acclimation, and 55, 46 and 39% with repeated injections of isoprenaline. activity of HK increased 16, 17 and 59% in response to training, cold-acclimation isoprenaline treatment, respectively. The increase in HK activity was significantly gr in the isoprenaline-treated rats than in the cold-acclimated ($p < 0.01$) or trained rats (p No changes were found in the activities of LDH and PFK. No changes were detect protein concentration of the cell-free homogenates of the gastrocnemius muscles.

Cold tolerance and swimming time tests

When placed at −18°C in groups of 4 the rats were able to maintain their body tempe for several hours (Fig. 1). After 12 hours at this temperature the colonic temperatu creased significantly more in the control animals than in other groups ($p < 0.01$–p Student's t test). The cold-acclimated animals, on the other hand, were much more tol to cold than the trained ($p < 0.02$) or isoprenaline treated rats ($p < 0.05$) as shown by a smaller decrease in colonic temperature.

The swimming time was tested at 30°C with a tail load of 5% of body weight. The r in Fig. 2 show that the training program used significantly increased the swimming ti these animals ($p < 0.001$, Mann-Whitney U-test). Also the cold-acclimated animals sh a slight improvement in resistance to exercise ($p < 0.05$). However the isoprenaline ment did not prolong the swimming time.



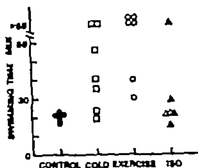


Fig. 1. Fig. 2.

Fig. 1. Colonic temperature changes at 10°C of control (●), cold-acclimated (□), trained (○) and isoprenaline-treated (daily injections of 0.3 mg/kg) rats (△). Each curve represents the mean ± S.E. of 4 rats.

Fig. 2. Swimming time of control, cold-acclimated, trained, and isoprenaline-treated (ISO) rats with a load of 5% of body weight in water at 30°C.

In the first swimming exercise at 30°C with a tail load of approximately 2% of body weight the mean colonic *temperature of the rats had been fallen by* 5.28 ± 0.34°C with the ... min exercise. In the 18th swim with a tail load of approximately 3.5–4% of body weight the fall was significantly smaller (2.96 ± 0.31 C, p 0.001).

Discussion

The lower body weight and the higher heart weight of the exercised and cold-acclimated rats in this investigation agree with many previous reports of studies (e.g. Smith 1962, ... et al. 1973, Hamilton and Ferguson 1972). An increase in the weight of ISBAT has been reported on cold-acclimated and on isoprenaline-treated rats (LeBlanc *et al.* 1972). In this study the swimming exercise used also increased the weight of ISBAT. This increase, however, may be due to repeated falls of body temperature caused by swimming in water at ...°C, and thus corresponds the cold-acclimation produced by repeated exposures of animals to cold (Tarkkonen 1971). The very marked increase in the heart weight produced by repeated injections of isoprenaline has been reported earlier (Stanton, Brenner and Mayfield 1969, LeBlanc *et al.* 1972). This increase is due to a great extent to the increased water content of the heart tissue (Stanton *et al.* 1969) as is also seen by the decreased protein concentration *of the heart muscle homogenates. In this study only the isoprenaline* treatment increased the weight of the adrenals significantly. This could indicate that this treatment was the most stressful one. In contrast to findings in the literature (Fröberg, Östman and Sjostrand 1972, Hearn and Vainio 1956, Smith 1962) the cold-acclimated or the trained rats did not show any increase in adrenal weight. This may be due to the very heavy adrenals of the rat strain used, compared with values presented by other authors (e.g. Hearn and Vainio 1956, Froberg *et al.* 1972).

Regular strenuous training increases the aerobic capacity of skeletal and cardiac m as is seen by the increased content of mitochondrial enzymes (Holloszy 1973). How similar changes in mitochondrial enzymes also are found with cold-acclimation (Ha 1963 Hamilton and Ferguson 1972). Accordingly in this study all the changes in ae enzymes in heart, as in skeletal muscle, were very similar in the trained as in the c acclimated rats. In the heart muscle these increases are due in part to increased pr content of the muscle, rather than to the increased enzyme activity as is seen by the incr protein concentration of heart muscle homogenate. Changes in the anaerobic enzym cold-acclimated and in trained rats also resemble each other whenever they are det (Hannon 1962, Lamb *et al.* 1969 Hannon and Vaughan 1960, Gollnick *et al.* 1973 this study significant changes (increase) were observable only in the activity of HK. T changes were detected both in heart and in skeletal muscle, and the changes were the in both cold-acclimated and in trained rats. In addition, both these treatments signific prolonged the swimming time and improved the cold resistance.

The results of LeBlanc *et al* (1972) show that repeated injections of rats with isopre improves the cold resistance. The present results show that this improvement can be acco ed for by the increased heat production capacity of the skeletal muscle and brown f is seen by the increase in the activities of aerobic enzymes in gastrocnemius muscle a the increased weight of ISBAT On the other hand the enzyme picture in heart muscle o isoprenaline treated rats is very different from those of other groups. The decreased ac of the aerobic enzymes can be accounted for by the low protein concentration of the homogenate, due to increased water content of the cardiac tissue (Stanton *et al.* 1 However the fact that the ratio of anaerobic to aerobic enzymes is strongly anaerol the isoprenaline-treated rats, suggests that this treatment really shifts the heart m metabolism to anaerobic pathways. It is known that isoprenaline reduces systemic a blood pressure (and possibly reduces coronary perfusion pressure) at a time when rate and myocardial oxygen demands are maximally elevated. This could result in rep bouts of myocardial hypoxia which could shift the metabolism of myocardium from ae to anaerobic pathways. Hypoxia has been suggested as one probable explanation of iso aline-induced cardiomegaly (Stanton *et al* 1969), seen also in the present results. Mor the poor results of isoprenaline-treated rats in the swimming time test might be due t failure of the heart.

As stated above, the metabolic changes in muscle metabolism as a result of stre training resemble very closely the changes produced by cold-acclimation or even by rep injections of rats with isoprenaline. In addition Kraus and Kirsten (1969) have sugg that corresponding enzymatic changes can be seen in mitochondria after thyroxine trea as well The possibility that the muscular shivering in cold corresponds to the mus work of training and thereby is responsible for the metabolic changes found, can be exc on the basis that shivering in the cold soon disappears (Smith 1962), and that similar bolic changes can be found in cold-exposed poikilothermic animals which do not (Lagerspetz, Harri and Okslahti 1974). Furthermore, the metabolic changes induce isoprenaline treatment or by hyperthyreoidism do not require either muscular work or On the other hand, the swimming exercise used caused a marked fall in body temper

ns. It is thus possible that the metabolic changes produced by the training program are result of repeated falls in body temperature, rather than from muscular work per se. s possibility is improbable as the only reason, because very similar metabolic changes e been detected with strenuous exercise runs on a treadmill (Holloszy 1967 Lamb al. 1969). Moreover Kraus and Kirsten (1969) found that a treadmill exercise induced tabolic changes in the myocardium and in the liver similar to those induced by a swim- g exercise even in a much colder water temperature (25 C).

n conclusion: either increased muscular activity or cold are not necessarily required for metabolic changes of skeletal muscle. An alternate explanation is the increased metabolic ulation by catecholamines. It is well known that the increased release of noradrenalin the cold sensitizes the animals to the calorigenic action of this amine (Janský 1973). also is known that increased release of catecholamines is associated with physical training Häggendal 1971), and a suggestion has been made that trained athletes may be more sitive to the metabolic effects of adrenalin and noradrenalin (Johnson and Rennie 1974). peated injection of rats with isoprenaline mimics the repeated stimulation of β-adreno- ceptors by natural catecholamines, and also leads to sensitization of β-receptors (LeBlanc et al. 1972). Furthermore, the unpublished results from our laboratory (Harri and Tirri) dicate that increased sympathetic activity or a mental stress (an electric shock connected with water drinking) can produce metabolic changes in the heart muscle, very similar to those scribed above. It is thus reasonable to assume that all the situations which include pro- nged stimulation of adrenergic β-receptors result in intensification of tissue metabolism, mainly of aerobic pathways. It can be concluded that the metabolic changes induced e.g. by physical training or by cold acclimation are not necessarily specific indicators for these treatments.

That improved physical performance is associated with the metabolic changes induced by strenuous physical training is well known, but whether or not the metabolic changes resulting from other stresses demonstrate physical performance is another question. In this study cold acclimation prolonged the swimming time of rats. However the body temperature of the rats fell during the test. According to Dawson, Roemer and Horvath (1970) cold acclimation extends the swimming time by slowing the rate of body cooling. This may be the reason for the improved swimming time of the cold-acclimated rats in the present study. Unfortunately the ambient temperature also influences exercise tolerance in air. In fact cold-acclimated rats tolerate running exercise better at a cold temperature while control rats tolerate it better at room temperature (Dieter Altland and Highman 1969 1970), leaving the question of improved physical performance in cold-acclimated animals still unsolved. In accordance with the results of LeBlanc *et al.* (1972) the isoprenaline treatment did not extend swimming time despite the increased aerobic capacity of skeletal muscle. This demonstrates that the energy metabolism of muscle as such does not necessarily give evidence about the physical fitness. This assumption is supported by the results of Barnard and Peter (1969), which show that although HK is very responsive to exercise, no relationship exists between HK activity of skeletal muscle and running endurance.

On the other hand, in the present results, cold tolerance was better in the trained rats

than in the controls. This improvement might be due to the repeated falls of body tempera- tures caused by the swimming. The results of the isoprenaline treatment, which did not induce changes in body temperature while increasing cold tolerance, indicate that neither cold environment nor a fall in body temperature are required for the improvement of cold tolerance. It can be concluded that all the situations which include intensification of tissue metabolism, mainly of aerobic pathways, result in improved cold tolerance. The fact that improved cold tolerance also results from treadmill exercise further supports this concept (Strømme and Hammel 1967).

Swimming is a geenrally used method in exercising rats. In these experiments the water temperature has been varied between 25 C and 33 C (Kraus and Kirsten 1969 25 C, Hearn and Wainlo 1956, 32°C Arcos *et al* 1968, 33 C Gould and Rawlinson, 1959, 27–30°C) All the temperatures used cause more or less marked changes in body temperature of rats (Dawson *et al* 1970) To what extent these falls have influenced the results obtained is not known. In future swimming experiments it is necessary to use a water temperature of 37°C (Dawson *et al* 1970) if the aim of the experiment is to measure the changes produced by physical activity only rather than those produced by cooling of the body and physical activity together

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References

ADAMS, T and E. J HEBERLING, H man physiological responses to a standardized cold stress as modified by physical fitness. *J appl. Physiol* 1958. *13* 226–230.

ARCOS, J C., R. J SOHAL, S.-C. SUN M F ARGUS and G E. BURCH, Changes in ultrastructure and respiratory control in mitochondria of rat heart hypertrophied by exercise. *Exp. Mol. Pathol.* 1968 49–65

BARBASHOVA, Z., *Acclimatization to Hypoxia and its Physiological Mechanisms.* Acad. Sci. USSR Moscow–Leningrad.

BARNARD R. J and J B. PETER, Effect of training and exhaustion on hexokinase activity of skeletal muscle *J appl. Physiol.* 1969 *27* 691–695

BASS, A., D BRDICZKA, P EYER, S. HOFER and D PETTE, Metabolic differentiation of distinct muscle types at the level of enzymatic organization *Europ J Biochem.* 1969 *10.* 198–206.

BOSTRÖM, S., M FAHLÉN, Å. HJALMARSON and R. JOHANSSON Activities of rat muscle enzymes after exercise. *Acta physiol. scand.* 1974 *90* 544–554

DAWSON, C. A., R. B. ROEMER and S. M HORVATH Body temperature and oxygen uptake in warm- and cold-adapted rats during swimming. *J appl Physiol* 1970. *29* 150–154.

DIETER, M P P D ALTLAND and B HIGHMAN, Exercise tolerance of cold-acclimated rats. Serum liver enzymes and histological changes. *Canad J Physiol. Pharmacol.* 1969 *47* 1025–1031

DIETER, M P P D ALTLAND and B HIGHMAN Tolerance of unacclimated and cold-acclimated rats to exercise in the cold serum, red and white muscle enzymes and histological changes. *Canad. J Physiol. Pharmacol.* 1970. *48* 723–731

EARL, D. C. N and A. KORNER, The isolation and properties of cardiac ribosomes and polysomes. *Biochem. J* 1965. *94.* 721–

FRÖBERG, S. O., L ÖSTMAN and N O SJÖSTRAND, Effect of training on esterified fatty acids and carnitine in muscle and on lipolysis in adipose tissue in vitro *Acta physiol. scand.* 1972. *86.* 166–174.

GOLLNICK, P D R. B. ARMSTRONG, B. SALTIN, C W SAUBERT IV W L. SEMBROWICH and R. E. SHEPHERD Effect of training on enzyme activity and fiber composition of human skeletal muscle. *J appl. Physiol.* 1973. *34* 107–111

...NCE, P. D. and L. HERMANSEN, Biochemical adaptations to exercise: Anaerobic metabolism. Pp. ... in Wilmore, J. H. (ed.): *Exercise and sport sciences reviews*, Academic Press, New York–London ..., Vol. I.

...LA, H. K. and W. A. RAWLINSON, Biochemical adaptations as response to exercise. I. Effect of swimming on the levels of lactic dehydrogenase, malic dehydrogenase and phosphorylase in muscles of 8- and 15-week-old rats. *Biochem. J.* 1959 *73*, 41–44.

...GREN, J., Role of circulating noradrenaline and adrenaline. Pp. 119–126. In Pernow, P. and Saltin, ... (eds.): *Muscle metabolism during exercise*. Plenum Press, New York–London, 1971.

...ERY, M. J. and J. H. FERGUSON, Effects of exercise and cold acclimation on the ventricular and skeletal muscles of white mice (*Mus musculus*)—I. Succinic dehydrogenase activity. *Comp. Biochem. Physiol.* 1972. *43A*, 815–824.

...NON, J. P. Current status of carbohydrate metabolism in the cold-acclimatized mammal. *Fed. Proc.* ... *22*, 856–861.

...NON, J. P. and D. A. V. DORAN, Effect of prolonged cold exposure on the glycolytic enzymes of liver ...al muscle. *Amer. J. Physiol.* 1960. *198*, 375–380.

...LL, G. R. and W. W. WAINIO, Succinic dehydrogenase activity of the heart and skeletal muscle of ...ccined rats. *Amer. J. Physiol.* 19... *185*, 348–350.

...NING, E. J. and T. ADAMS, Relation of changing levels of physical fitness to human cold acclimatization. *J. appl. Physiol.* 1961. *16*, 226–230.

...LLOSZY, J. O., Biochemical adaptations in muscle. Effect of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J. biol. Chem.* 1967. *242*, 2278–2282.

...LLOSZY, J. O., Biochemical adaptations to exercise: aerobic metabolism. Pp. 45–71. In Wilmore, J. H. (ed.): *Exercise and sport sciences reviews*, Academic Press, New York–London, 1973, Vol. 1.

...LLOSZY, J. O., L. B. OSCAI, P. A. MOLÉ and L. J. DON, Biochemical adaptations to endurance exercise in skeletal muscle. Pp. 51–61. In Pernow, B. and Saltin, B. (eds.): *Muscle metabolism during exercise*. Plenum Press, New York–London, 1971, Vol. 11.

...ACE, L., Non-shivering thermogenesis and its thermoregulatory significance. *Biol. Rev.* 1973. *48*, 85–132.

...NON, R. and M. RIVERS, Athletic training and metabolism. *New Scientist* 1974. *21*, 585–587.

...ATON, W. R., The effect of repeated daily exposure to cold and of improved physical fitness on the metabolic and vascular response to cold air. *J. Physiol. (Lond.)* 1963. *157*, 209–220.

...MANN, H. and R. KOSTEN, Die Wirkung von körperlichem Training auf die mitochondriale Energieproduktion im Herzmuskel und in der Leber. *Pflügers Arch. ges. Physiol.* 1970. *320*, 334–347.

...ROWITZ, K. Y. H., M. N. E. HARRI and R. OHLMANN, The role of the thyroid in the temperature acclimation of the oxidative metabolism in the frog, *Rana temporaria*. *Gen. comp. Endocr.* 1974. *22*, 169–176.

...on, D. R., J. B. PETER, R. N. JEFFRESS and H. A. WALLACE, Glycogen, hexokinase, and glycogen synthetase adaptations to exercise. *Amer. J. Physiol.* 1969. *217*, 1628–1632.

...von AMERONGEN, K., Metabolic and circulatory aspects of tolerance to cold as affected by physical training. *Fed. Proc.* 1966. *25*, 1351–1356.

...BLANC, L. J. VALLIÈRES and C. VACHON, Beta-receptor sensitization by repeated injections of isoproterenol and by cold adaptation. *Amer. J. Physiol.* 1972. *222*, 1043–1046.

...WRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, Protein measurement with the Folin phenol reagent. *J. biol. Chem.* 1951. *193*, 265–275.

...CHS, S. Malic dehydrogenase from pig heart. Pp. 735–739. In Colowick, S. P. and N. O. Kaplan (eds.): *Methods in enzymology*. Academic Press, New York–London, 1955, Vol. 1.

...SON, L. B., The role of exercise in weight control. Pp. 103–123. In Wilmore, J. H. (ed.): *Exercise and sport sciences reviews*, Academic Press, New York–London, 1973, Vol. 1.

...RTIN, R. E. Cold acclimation—an altered steady state. *J. Amer. med. Ass.* 1962. *179*, 948–954.

...ERE, P. A., Citrate synthase. Pp. 3–19. In Lowenstein, J. M. (ed.): *Methods in enzymology*. Academic Press, New York–London, 1969, XIII.

...RTON, H. C., BRUNNER, O. and E. D. M...FIELD, Jr. Studies on isoproterenol-induced cardiomegaly in rats. *Amer. Heart J.* 1969. *77*, 72–80.

...SEMAN, S. R. and H. T. HAMMEL, Effect of physical training on tolerance to cold in rats. *J. appl. Physiol.* 1967. *23*, 815–824.

TARKOVEN, H., Effect of repeated short-term exposure on brown adipose tissue of mice. *Ann. Zool. Fennici* 1971. *8*, 434–439.

Acta physiol. scand. 1975. 95. 400–406

From the Department of Physiology University of Oulu and Department of Physiology University of Helsinki, Finland

Secretion of Anterior Pituitary Hormones in Man: Effects of Ethyl Alcohol

By

J LEPPÄLUOTO M RAPELI, R VARIS and T RANTA

Received 5 May 1975

Abstract

LEPPÄLUOTO, J., M RAPELI R VARIS and T RANTA. *Secretion of anterior pituitary hor in man Effects of ethyl alcohol* Acta physiol scand. 1975 95 400–406.

The possibility that previously described effects of ethyl alcohol on peripheral endocrine glands ar *mediated* via pituitary prompted this investigation on the effects of ethanol on anterior pituitary sec *Nine healthy male* subjects were given beverage containing ethanol (1.5 g/kg) or beverage alone per *randomized* cross-over study and plasma ACTH FSH GH LH and TSH were measured by specific *immunoassays up to 15 h* and the urinary levels of adrenaline and noradrenaline by fluorometry. A *blood LRF and TRF* test was also carried out n similar series of experiments. During the whole exper *there were no sign ficant* differences in the plasma levels of ACTH FSH and TSH or in the urinary *of adrenaline and noradrenaline* between ethanol treated and control subjects. Plasma FSH, LH and *responses to LRF and TRF* timulation wer also similar in alcohol treated and control subjects. P *ACTH values were high (113–270* pg/ml) both in control and ethanol experiment suggesting that the *jects experienced apprehension* toward the experiment Plasma GH level exhibited a non-sleep related in the late evening (from 0.4 ng/ml at 6 p m to 3 1 *ng/ml at 10 p m., p < 0.01)*. Thus increase was not after alcohol ingestion (p < 0.01). Plasma LH levels were *significantly lower after* 6 and 13 h in th treated subjects than n controls (65 *s.* 106 ng/ml p 0.01 and 74 *vs.* 121 ng/ml, *p* 0.05 respectiv Because ethanol had no effect n the rest ng level of plasma GH or on the LH response to LRF we co that ethanol exerts these effect on a suprapituitary site.

Some behavioral effects of moderate doses of ethyl alcohol in man are regarded as stim tory whereas most of the known endocrine effects *appear to be inhibitory* Ethanol produ water diuresis accompanied by decreased chlorine output, similar to that seen after inges of water (Eggleton and Smith 1946) Uterine motility and milk ejection in response to suckl was abolished by ethanol in lactating rabbits (Fuchs and Wagner 1963) This all is sugges of decreased release of vasopressin and oxytocin from hypothalamus.

There is evidence that alcohol might also inhibit the function of the pituitary-gonad a Ovulation in response to electrical stimulation of hypothalamus was completely blocked rabbits when alcohol was administered prior to stimulation (Saul 1959). In human subje ethanol does not appear to change the plasma LH concentration (Toro *et al* 1973), althou testosterone concentration has been demonstrated to be greatly decreased during the han over period (Ylikahri *et al* 1974)

nd radioiodine uptake in chronic alcoholics and in experimental animals after long
cohol treatment has been reported to be elevated (Murdock 1967), and the secretion
cocortical hormones increased after high or consecutive ethanol doses in man (Merry
arks 1969, Mendelson et al. 1971). The mechanism of these phenomena is unknown.
etermine if some of the endocrine effects of ethyl alcohol might be mediated via
alamus and the pituitary gland, we have studied the short term effects of ethanol
stration on the plasma concentration of ACTH, FSH, GH, LH and TSH in healthy
bjects.

Material and Methods

s. 9 healthy non-obese male medical students (21–25 yrs) volunteered this study. None had a history of heavy drinking and had not previously been exposed to repeated venipunctures or alcoholic studies. ... The experiments were carried out in 4 parts, 1–3 weeks apart from each other. Each experiment was begun at 6 p.m. on Friday and a slight meal was allowed 2–4 h before. 1.5 g of ethanol per kg was administered diluted to 100–400 ml of beverage. Controls received beverage only. Alcohol or beverage was taken per os between 6 and 9 p.m.

Exp. I five randomly elected subjects (group A) were given the alcohol regimen and the remaining (group B) served as controls. Blood samples were drawn from each subject at 6, 8, 10 and 12 p.m. The subjects slept at the institute and on the following morning blood samples were taken at 7 and 9 a.m. Exp. II was carried out in the same manner as exp. I, but group B was given ethanol and group A served as controls.

Exp. III group A was given ethanol and group B served as controls as in exp. I, but the first blood sample was taken at 8–10 p.m. Then 100 µg of LH releasing factor (LRF) and 200 µg of TSH releasing factor (TRF) diluted to 1 ml of saline were given ... The peptides were synthetized by Ferring, Malmö, Sweden. ... blood samples were taken 20 and 60 min later. Then the subjects slept at their homes and brought ... of morning urine to the institute in the following day.

Exp. IV was carried out as exp. III except that group B was given ethanol and group A served as controls.

Hormone determinations. Blood samples were collected into heparinized test tubes and kept at 4°C for ... h. The plasmas were separated by centrifugation and kept at −20 C before the determinations (2–4 ... the later). The plasma ACTH and GH concentrations were estimated by commercial radioimmunoassay kits (Amersham, England and Kabi, Sweden). The materials for the radioimmunoassay for FSH, LH and TSH were received from NIAMDD, USA. The plasma ACTH concentrations were expressed as pg/ml and GH as ng/ml against kit standards. FSH and LH were expressed as µg/ml of LER 907 standard and TSH as µU/ml of MRC standard 63/68. All the estimations were made in duplicate and in one run in order to avoid inter-assay variability (except ACTH). The intra-assay coefficient of variation calculated from all the duplicate estimations varied from 4 to 18 % in different assays. The inter-assay coefficient of variation in ACTH radioimmunoassay was 4.3 % at the dose level of 300 pg/ml (N = 4). Hormone concentrations were read graphically from a standard curve with percentage binding plotted against the logarithm of dose.

15 ml of 6 N HCl were added to 100 ml samples of urine and kept at −20°C before the estimation. Noradrenaline and adrenaline concentrations were estimated after aluminium oxide absorption in fluorometry.

Statistical analyses. Statistical comparisons between results obtained in various experimental groups were performed using a computer program built up by Sakar and Guilleman (1962). In brief, homogeneity of variances within each treatment was first tested according to Bartlett. In the absence of significant inhomogeneity Duncan's test for multiple comparisons were used, in other cases non-parametric test of Wilcoxon. P values smaller than 0.05 were regarded as significant.

Results

General findings. All the test subjects experienced some apprehension toward venipunctures and their ability to tolerate the effects of ethanol, especially in first experiments. The following behavioral changes were observed after ingestion of ethanol: rapid and loud speaking, ... agitation, attempts to leave the institute. One subject in the first experiment experi-

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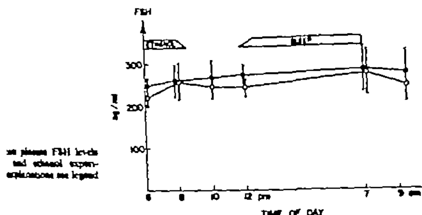
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The possibility that previously described effects of ethyl alcohol on peripheral endocrine glands mediated via pituitary prompted this investigation on the effects of ethanol on anterior pituitary Nine healthy male subjects were given beverage containing ethanol (1.5 g/kg) or beverage alone randomized cross-over study and plasma ACTH FSH GH LH and TSH were measured by specif immunoassays up t 15 h and the urinary levels of adrenaline and noradrenaline by fluorometry bined LRF and TRF test was also carried out in similar series of experiments. During the hole exp there were no significant differences in the plasma levels of ACTH FSH and TSH or in the urinar of adrenaline and noradrenaline between ethanol treated and control subjects. Plasma FSH, LH responses to LRF and TRF stimulation were also similar in alcohol treated and control subjects. ACTH values were h gh (113–270 pg/ml) both in control and ethanol experiment suggesting that jects experienced apprehension toward the experiment Plasma GH level exhibited a non-sleep relate in the late evening (from 0.4 g/ml at 6 p.m. to 3 1 ng/ml at 10 p.m., $p < 0.01$). This increase was after alcohol i gestion ($p < 0.01$) Plasma LH levels were s gnificantly lower after 6 and 13 h in treated subjects than n controls (65 *vs* 106 ng/ml, p 0.01 and 74 *vs.* 121 ng/ml, $p < 0.05$ respect Because ethanol had no effect on the resting level of plasma GH o on the LH response to LRF we that ethanol exerts these effect on suprapituitary site.

Some behavioral effects of moderate doses of ethyl alcohol in man are regarded as stim tory whereas most of the known endocrine effects appear to be inhibitory Ethanol prod water diuresis accompanied by decreased chlorine output similar to that seen after inges of water (Eggleton and Smith 1946). Uterine motility and milk ejection in response to suck was abolished by ethanol in lactating rabbits (Fuchs and Wagner 1963). This all is sugge of decreased release of vasopressin and oxytocin from hypothalamus.

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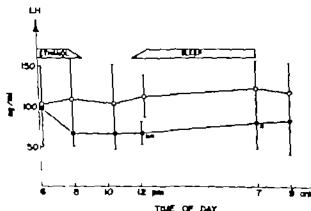


...se plasma FSH levels ... and ethanol experi... explanations see legend

...246 to 277 ng/ml in ethanol treated subjects and no significant diurnal or alcohol ...were seen (Fig. 3). In LRF and TRF test plasma FSH presented a significant rise ..., Duncan's test) at 20 and 60 min both in control and ethanol treated subjects but the ... between the rises was not significant (Fig. 6 a).

...*LH concentration.* Plasma LH in control subjects varied from 104 to 121 ng/ml ...he experiment and did not show any diurnal fluctuation (Fig. 4). In ethanol treated ... LH levels were generally lower from 8 p.m. and the decrease was significant at ...and 7 a.m. (65 *vs.* 106 ng/ml, p 0.01 and 74 *vs.* 121 g/ml, p 0.05 Wilcoxon test). ...and TRF stimulation plasma LH rose in 20 min from 100 to 245 ng/ml in controls ... Duncan test) and from 90 to 297 ng/ml (p 0.01) in alcohol treated subjects. These ...s in responses to LRF were similar in control and alcohol treated subjects (p > 0.05, ...).

...*TSH concentration.* Plasma TSH varied from 3.7 to 4.3 μU/ml in control and from ...4 μU/ml in ethanol treated subjects. No significant diurnal or ethanol effect was ... p 0.05 Duncan test). In TRF stimulation test TSH rose in 20 min from 4.5 to ...U/ml (p 0.01) in control and from 5.4 to 16.8 μU/ml (p 0.01) in ethanol treated



...Mean plasma LH levels ...trol and ethanol experi... For explanations see legend ...1.

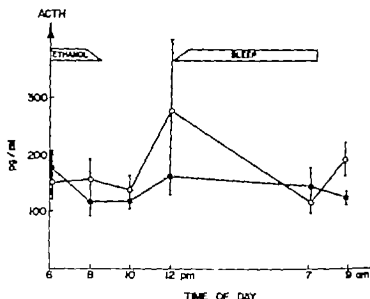


Fig. 1 Mean plasma ACTH levels of 9 normal men in control (open) and ethanol (black circles) experiment. Vertical bars denote ±1 S.E. The asterisks and control circles indicate values significantly (one: p < 0.05, two: p < 0.01) different than the 6 p.m. value, inside ethanol circles significantly different than the respective control value. The absence of the asterisk denotes no significant difference (p > 0.05)

enced nausea and vomiting. On the following morning subjective expressions of the hangover were present

Plasma ACTH concentration From the beginning of the experiment the plasma ACTH levels were high both in control and ethanol treated subjects the respective mean values varied from 124 to 270 pg/ml and from 113 to 170 pg/ml, (Fig. 1). Variances were heterogeneous (Bartlett), and in non-parametric test (Wilcoxon) no significant differences between control and ethanol treated subjects were found

Plasma GH concentration. In control subjects plasma GH rose from 0.4 ± 0.1 ng/ml (mean ± S.E.) at 6 p.m. to 3.1 ± 1.3 ng/ml at 10 p.m. ($p < 0.01$ Wilcoxon test), and then returned to initial levels (Fig. 2) In ethanol treated subjects the plasma GH level varied from 0.4 to 0.8 ng/ml during the whole experiment and was significantly ($p < 0.01$) lower at 10 p.m. than in controls at 10 p.m. (0.6 vs 3.1)

Plasma FSH concentration. Mean plasma FSH varied from 218 to 264 ng/ml in control

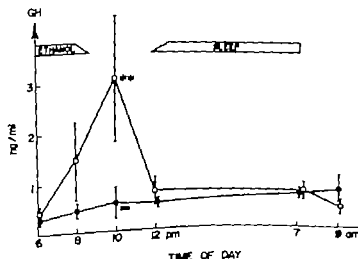


Fig. 2 Mean plasma GH levels in control and ethanol experiment. For explanations see legend to Fig. 1

found to block ovulation (Saul 1954) as well as the late decrease of plasma testosterone was by Ylikahri *et al.* (1974). It is noteworthy that we did not find diurnal fluctuation in plasma LH. It is possible that we ha e missed the LH discharges reported to occur in the morning (Nankin and Troen 1972).

There is no previous information about the effects of ethanol on plasma FSH and, according to our results, alcohol does not seem to have any effect on plasma FSH up to 15 h. Our results also failed to reveal diurnal fluctuation in plasma FSH levels.

In our combined LRF and TRF test plasma LH and FSH responses to LRF were similar in alcohol and control experiments. This suggests that the effects of ethanol on inhibition of LH secretion, described here, occurs at a suprapituitary site.

Control subjects exhibited a non-sleep related burst of GH in the evening (at 10 p.m.). The nocturnal rise in GH secretion generally occurs during the early sleep (Quabbe *et al.* 19.., but according to our results, GH is also secreted in the late evening in wakeful state. This finding was confirmed by a recent work (Plotnick *et al* 1975). The observed evening rise of GH was however not seen after ethanol administration. Thus alcohol appears to inhibit the stimulated GH secretion, but has no effect on resting levels. Our finding is in agreement with that made by Arky and Freinkel (1964), who demonstrated impaired GH responses to ethanol induced hypoglycemia in 2 patients.

In the present study plasma TSH levels showed no diurnal fluctuation and were similar in control and ethanol experiments as were the responses to TRF. In previous studies there is no information about plasma TSH levels, but elevated radioiodine uptakes have been demonstrated in chronic alcoholics and in rats after long term ethanol treatment (Murdock 67). These results suggest that alcohol might have a direct stimulatory effect on the thyroid, and possible changes in plasma TSH might occur in late phase.

Previous reports about the effects of ethyl alcohol on the pituitary-adrenocortical axis concern the glucocorticoidal responses only. Non-alcoholic individuals respond to high dose ethanol with a rise of plasma cortisol (Merry and Marks 1969). After moderate alcohol doses, Kissin *et al* (1958) and Perman *et al* (1961) did not find adrenocortical stimulation in a short-time experiment. On the other hand, long-term alcohol ingestion is associated with adrenocortical activation, which is at the greatest during vegetativ symptoms, such as gastric pains (Mendelson *et al.* 1971). In the present study the plasma ACTH concentrations were clearly elevated at the beginning of the experiments, both in control and ethanol runs. These high plasma ACTH levels were evidently due to enhanced emotional reactions of the test subjects, who were for the first time exposed to this type of an experiment. Increased cortisol secretion in control experiments has been earlier observed in "first-time" participants by Shabam *et al.* (1958) and by Sutton and Casey (1975). This anxiety reaction interfered with the interpretation of our ACTH results in this respect. We emphasize that ethanol administration did not produce a tranquillizing effect that would be reflected in the fall of ACTH secretion.

Collection of urine samples for measurement of adrenaline and noradrenaline secretions was carried out after the shorter and later experiments, when the anxiety reactions were absent. The urine levels of adrenaline and noradrenaline were normal and similar both in control and ethanol experiments. In previous work, increased urinary adrenaline secre-

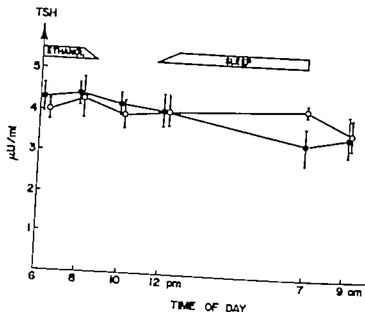


Fig. 5. Mean plasma TSH levels in control and ethanol experiment. For explanations see legend in Fig. 1

subjects (Fig. 5 and 6 c) The increases in responses to TRF were again similar in control and ethanol treated subjects (p 0 05)

Urine catecholamine concentrations Urinary noradrenaline level in control subjects was 37 ± 3 ng/ml at 7 a.m and adrenaline level 4 1 ± 0 7 ng/ml After ethanol administration the respective noradrenaline and adrenaline levels were 38 ± 2 and 3 2 ± 1 4 ng/ml ($p > 0.05$).

Discussion

The most prominent effect of alcohol in the present study was the decrease of plasma LH after 6 h from the beginning of the experiment Toro and his coworkers (1973) failed to find any changes in plasma LH levels after ethanol administration. This is not necessarily in disagreement with our results, because in latter study the observation period was only 3 h. However in blood samples obtained 12 h after ethanol ingestion a significant decrease in plasma testosterone with a compensatory rise in plasma LH was recently demonstrated (Ylikahri *et al* 1974) At the moment we cannot give an explanation for this divergence. Our low LH values are in better agreement with the previous animal studies, in which ethanol

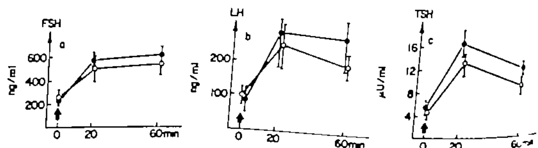


Fig. 6. Mean plasma FSH (6 a), LH (6 b) and TSH (6) LRF nd TRF test. Open circle = control experiment and black circle = ethanol experiment. The arrow ndicates jection of 100 µg of LRH and 200 µg of TRF

All the increments after 20 min significant (p 0.01), but th differences bet een control and ethanol experiment not ($p > 0.05$).

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From the Department of Toxicology, University of Uppsala, Sweden

on the Renal Uptake of Vitamin D_3 in the Mouse and the Quail

By

Lennart Dencker and Hans Tjälve

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Abstract

r, L. and H. Tjälve. *Studies on the renal uptake of vitamin D in the mouse and the quail.* Acta physiol. scand. 1975, 95 407–416.

uptake of labelled vitamin D_3 was studied in the mouse and the quail. Upon the administration of vitamin D_3 autoradiographic experiments showed specific accumulation of radioactivity in the tubuli of the mouse kidney. This was still obvious 18 days after the administration. In the quail, on the other hand, the uptake in the kidney did not exceed the level of the blood. In the mouse there was an increase in the amount of steroid in the kidney after the injection of vitamin D_3. maximum reached 24 hours after the administration. The amount of steroid which accumulated in the kidney was proportional to the injected dose of the vitamin—from doses at 4.8 ng to 4.8 µg. Column chromatography showed that most of the renal vitamin D_3 was present in non-metabolized form. Cellular fractionation showed that most radioactivity in the kidney was present in the mitochondrial and microsomal fractions. Upon sonication of the fractions most radioactivity was still bound to these particles.

Key words: Vitamin D_3, kidney, proximal tubuli, autoradiography, mouse, quail

s in recent years indicate that the kidney plays an important role in the metabolism of vitamin D to more active metabolites, (for a review see *e.g.* Kodicek 1974). In addition the kidney is considered to be an important site of action for the vitamin, although most of renal effects are still obscure (Gekle *et al.* 1971, Puschett *et al.* 1972 a, b).

In a preliminary autoradiographic study a very selective accumulation of radioactivity was observed in the kidney after the administration of labelled vitamin D in mice (Dencker and Tjälve 1973). In the present study more data on the renal uptake of vitamin D are reported. Mice and quails are used as experimental animals.

Material and methods

Isotopes

) cholecalciferol (^{14}C-vitamin D_3), spec. activity 32.2 mCi/mmol, (1α, 2α-^{3}H)-cholecalciferol (^{3}H-vitamin D_3), spec. activity 8.2 Ci/mmol and (26 (27)-^{3}H)-25-hydroxycholecalciferol (^{3}H-25-hydroxyvitamin D_3), spec. activity 6.9 Ci/mmol were obtained from the Radiochemical Centre, Amersham, England.

Animals

Mice of the C57BL strain (body weight 20–25 g) were used. The mice were fed either a complete diet or a vitamin D-deficient radiotogenic pellet diet of a composition similar to that described by

tion after ethanol ingestion has been reported at 4 h (Perman 1958). Our urine collection occurred at 15 h, and hence, the comparison between his and our results is difficult.

Our subjective observations suggest that administration of moderate doses of ethanol may have stimulated behaviour in healthy male subjects. In addition, ethanol resulted in decreased secretion of LH and GH but did not effect on the secretion of FSH, TSH adrenaline or noradrenaline. A normal response of LH to LRF and a normal resting level of GH suggests that ethanol exerts this inhibitory effect on hypothalamic or suprahypothalamic structures.

This study was supported by a grant from the Alcohol Research Foundation, Finland. We are grateful to the National Pituitary Agency NIAMDD U.S.A. for providing material for human FSH, LH and TSH radioimmunoassays. We also thank Dr Jan Mulder for supplying synthetic LRF and TRF (Ferring Ltd, Sweden).

References

ARKY R. A and N FREINKEL, The response of plasma human growth hormone to insulin and ethanol-induced hypoglycemia in two patients with isolated adrenocorticotropic defect *Metabolism* 1964 *13* 547 550.

EGGLETON M G and I G SMITH The effect of ethyl alcohol and some other diuretics on chloride excretion in man *J Physiol.* (Lond) 1946. *104* 435–442.

FUCHS, A R and G WAGNER, The effect of ethyl alcohol on the release of oxytocin in rabbits *Acta endocr* (Kbh) 1963 *44* 593–605.

KRUSIUS, F E., K. O VARTIA and O. FORSANDER, Experimentelle Studien über die biologische Wirkung von Alkohol. II Alkohol und Nebennierenrinden-Funktion. *Ann. Med. exp Fenn.* 1958. *36* 424-431.

MENDELSON, J H M OGA A and N K MELLO, Adrenal function and alcoholism. I Serum cortisol *Psychosom. Med.* 1971 *33* 145–157

MERRY J and V MARKS, Plasma-hydrocortisone response to ethanol in chronic alcoholics. *Lancet* 1969 *1* 921 923

MURDOCK, H. R J Thyroidal effect of alcohol. *Quart J Stud. Alc* 1967 *28* 419–423.

NANKIN, H R and P TROEN Overnight patterns of serum luteinizing hormone in normal men. *J. clin Endocr* 1972. *35* 705–710.

QUABBE, H J E. SCHILLING and H HELGE, Pattern of growth hormone secretion during a 24-hour fast in normal adults. *J clin. Endocr* 1966. *26*. 1173–1177

PERMAN E. S., The effect of ethyl alcohol on the secretion from the adrenal medulla in man. *Acta physiol scand.* 1958. *44* 241 247

PERMAN, E. S., Observations on the effect of ethanol on the urinary excretion of histamine, 5-hydroxyindole acetic acid catecholamines and 17-hydroxycorticosteroids in man *Acta physiol scand.* 1961 *51* 62–79

PLOTNICK, L. P R. G THOMPSON A KOWARSKI, L. deLACERDA, C. J MIGEON and R. M BLIZZARD, Circadian variation of integrated concentration of growth hormone in children and adults. *J clin Endocr* 1975. *40* 240–247

SAKIZ, E. and R. GUILLEMIN, Utilisation de routine d un grand ordinateur dans l analyse mathématique des résultats en biologie expérimentale. *C. R Acad Sci* (Paris) 1962. *254* 3743–3745.

SABSHIN, M D. HAMBURG, R. GRINKER H PERSKY H BASOWITZ, S. KORCHIN and J CHEVALIER, Significance of preexperimental studies in the psychosomatic laboratory *Arch. Neurol. Psychiat* (Chic) 1957 *78* 207–219

SAUL, G Blockade of ovulation in the rabbit by intoxicating doses of ethyl alcohol. *Anat Rec* 1955. *122* 332.

SUTTON, J. R. and J H. CASEY The adrenocortical response to competitive athletics in veteran athletes. *J clin. Endocr* 1975. *40* 135–138.

TORO, G R. C. KOLODNY L. S. JACOBS, W H MASTERS and W H DAUGHADAY Failure of alcohol to alter pituitary and target organ hormone levels. *Clin Res* 1973 *21* 505

YLIKAHRI, R., M. HUTTUNEN, M. HÄRKÖNEN, U SEUDERLING, S. ONIKKI, K. L. KARONEN and H. ADLERCREUTZ, Low plasma testosterone values in man during hangover *J Ster Biochem.* 1974. *5*. 655-658

phase was then separated after adding 2 volumes of water. The aqueous methanol residue was further extracted with 1 volume of chloroform. The combined chloroform-phases were evaporated to dryness under nitrogen and immediately redissolved in 0.2 ml n-hexane-chloroform 35 : 65 (v/v) to be used for column chromatography. The column chromatography was performed at 4 C with Sephadex LH-20 (obtained from Pharmacia Fine Chemicals, Uppsala, Sweden) (Holick and DeLuca 1971) using a column (…) equilibrated with n-hexane-chloroform 35 : 65 (v/v). The same solvent system was used for elution. All fractions were collected. The fractions were evaporated with air to dryness in liquid scintillation vials and counted in a Packard liquid scintillation spectrometer after the addition of scintillation fluid (PPO 4.25 g POPOP/liter toluene). Correction for quenching was made by the use of an external standard. ³H-vitamin D3, ¹⁴C-vitamin D3 and ³H 25-hydroxyvitamin D3 were used as references to determine their elution.

Counting

The distribution of radioactivity in the kidney after the injection of ³H-vitamin D3 was determined in mice fed the complete diet. Each mouse in the first series received 0.1 μCi (4.8 … g = 1.22 10⁻ …) ³H-vitamin D3 dissolved in 25 μl abs. ethanol. In the other series 0.1 μCi ³H-vitamin D3 was mixed with non-labelled vitamin D3 and injected (dissolved in 25 μl ethanol). Thus in the second series each mouse received 48 ng = 1.22 10⁻¹ nmol vitamin D3, in the third series 0.48 μg = 1.22 nmol vitamin D3, in the fourth series 4.8 μg = 12.2 10⁰ nmol vitamin D3 and in the fifth series 48 μg = 1.22 10² nmol vitamin D3. Three mice in each series were killed respectively 1 h, 2 h, 4 h, 8 h, 24 h and 48 h after the injection of vitamin D3. They were perfused as described above (see under Column chromatography) and their kidneys were removed. These were then dissolved in Soluene and the radioactivity was determined by liquid scintillation counting. With the knowledge of the specific activity of the parent vitamin the determinations per minute in the samples were converted to the absolute amount (pmol) of steroid in the kidney.

Cellular fractionation

… mice (fed the vitamin D-deficient diet) were injected i.v. with 0.1 μCi (4.8 ng) ³H-vitamin D3 (dissolved in 25 μl dimethylsulfoxide). Six mice were killed at 24 h and six at 8 days after having first been perfused. 12 mice were injected in the same way with 0.1 μCi ³H-vitamin D3 together with 4.8 μg non-labelled vitamin D3 and were killed at the same survival intervals as above (6 mice at each interval) after having been perfused. The kidneys were removed and manually homogenized in 10 ml 0.25 M sucrose in a Potter-Elvehjem glass tissue grinder with teflon pestle (Kontes Ltd., N.J. USA). The tissue suspensions were filtered through a double layer of gauze. Cellular fractionation was performed by centrifugation in 0.25 M sucrose in an M.S.E. high speed centrifuge (M.S.E. Ltd., London, England) first at 650 × g for 10 min to obtain the nuclear fraction, then at 16,000 × g for 20 min to obtain the mitochondrial fraction and finally at 105,000 × g for 1 h to obtain the microsomal and supernatant fractions (r_{av} 109.7 mm). Microscopical examination of the nuclear fraction showed that it was practically free of contamination with unbroken cells and cellular debris. The radioactivity of the fractions was determined by liquid scintillation counting after dissolution in Soluene as described above.

In an additional experiment two mice were injected i.v. with 0.1 μCi ³H-vitamin D3 together with 4.8 μg non-labelled vitamin D3 and were killed after 24 h. The kidneys were removed after perfusion and the mitochondrial and microsomal fractions were isolated as described above. These fractions were then diluted in 5 ml sucrose and subjected to sonication in a Branson Sonifier at 5A for 4 × 2 min (interruptions for cooling) in an ice bath. The sonified fractions were then centrifuged at 105,000 × g for 1 h and the radioactivity in the resulting pellets and supernatants was determined by liquid scintillation counting as described above.

Results

Whole body autoradiography

General. After the injection of ¹⁴C-vitamin D3 there was no difference in the distribution of radioactivity between the mice fed the complete diet and those which received the vitamin D-deficient diet. At the studied survival intervals there was nor an obvious difference between the i.v. and the s.c. injected animals. In the description below no distinction will therefore be made between the different groups of animals.

Steenbock and Black (1925) and Numerof *et al* (1955). The diets were supplied by AB Astra-Ewos, Södertälje, Sweden. The vitamin D-deficient diet consisted of (per cent of weight): corn (ground) 75, wheat gluten 20 $CaCO_3$ 3 Na Cl 1 and a vitamin mixture 1 The vitamin mixture had the following composition (per cent of weight)

Vitamin A 500 000 IE/g 0.200, thiamine 0 040, riboflavin 0.124 pyridoxine 0 050, Ca-pantothenate 45% 0.222, nicotinic acid 0.400, vitamin B_{12} 0.05% 0.400 vitamin K (menadione) 0 015, biotin 1 % 0.30 ascorbic acid 5 000, inositol 0.300, α-tocopherol 500 mg/g 0.840, choline chloride 50 „ 20 00, p-aminobenzoic acid 2.500, folic acid 0.005 and starch (protein-free) 69 604 The content of calcium and phosphorus in the diet was 1 16% and 0.27 „ respectively The mice fed this diet received it from their third week of age until used in the experiments 5–8 weeks later The serum calcium value of the mice fed the vitamin D-deficient diet was 8 4 ± 0.5 mg/ml (mean ± S.E. 7 determinations), while in the mice receiving the complete pellet diet the serum calcium concentration was 9 7 ± 0.2 mg/ml (mean ± S E., 7 determinations). The serum calcium was determined by a flame photometric method at the Department of Clinical Chemistry University Hospital, Uppsala.

Quails. One male and one female Chinese dwarf quail (Excalfactoria chinesis, b.w about 40 g) and a laying Japanese quail (Coturnix coturnix japonica, b.w about 140 g) were used. The birds were fed a complete pellet diet (Harald Forss & Co AB, Stockholm, Sweden).

Autoradiography

Whole body autoradiography Mice fed the complete diet, mice fed vitamin D-deficient diet and the quails were used in this investigation. The mice received ^{14}C-vitamin D either subcutaneously in the ro (dissolved in 25 μl dimethylsulfoxide) or intravenously in a tail vein (dissolved in 25 μl abs. ethanol). The quails received the vitamin subcutaneously dissolved in 25 μl dimethylsulfoxide. In the complete diet group three male mice were injected i.v. receiving 1 6 μCi (19 μg) ^{14}C-vitamin D_3 each and then killed after 5 min, 4 h and 24 h. Five mice were injected s.c., each animal receiving 1.0 μCi (12 μg) ^{14}C-vitamin D_3 and were then killed after 1 h (male), 4 h (male), 8 h (male), 4 days (female) and 18 days (female). Ten mice fed the vitamin D-deficient diet were injected s.c. receiving 0.5 μCi (6μg) ^{14}C vitamin D_3 each and were killed after 1 h (2 male mice), 8 h (2 male mice), 4 h (2 female mice), 4 days (1 female mouse), 8 days (2 female mice) and 18 days (1 female mouse). The Chinese dwarf quails were injected s.c. receiving 1 μCi (12 μg) ^{14}C vitamin D_3 each and were killed after 8 h (male) and 4 h (female). The Japanese quail was injected s.c. with 3.0 μCi (36 μg) ^{14}C vitamin D_3 and was killed after 4 days.

At the stated survival intervals the animals were anaesthetized with ether embedded in an aqueous gel of carboxymethyl cellulose, immersed in a mixture of solid CO_2 and hexane (−78°C) and subjected to autoradiography according to Ullberg (1954 1958). This procedure includes sectioning of the whole animals (20 μm thick sections) at −15 C in a microtome on tape (No 800, Minnesota Mining and Manufacturing Co. USA), freeze-drying of the sections at −15°C and autoradiography by apposition of the sections against X ray films (Structurix D7 Gevaert) The exposure (the exposure time being 8–28 months) was carried out at −15°C to avoid diffusion of lipid soluble material After the exposure time the sections were separated from the films. The films were developed in Gevaert G230 and fixed in Gevaert G305A.

Microautoradiography One mouse, fed the vitamin D-deficient diet was injected subcutaneously with 3 μCi (36 μg) ^{14}C vitamin D_3, and was killed 4 h after the injection by cervical dislocation. Pieces of the kidneys were quickly removed and frozen in isopentane cooled with liquid nitrogen. 5 μm thick sections of the kidneys were then taken in a cryostat (−15°C) in a dark room and placed directly on photographic plates (K2 and G5 Ilford). The exposure was carried out at −15°C for about 1 year After the exposure the freeze-sections (remaining on the plates) were fixed in formalin. The plates were developed for 3 min in Kodak D19 and fixed, and the sections were stained with haematoxylin-eosin and mounted under cover glass, still on the plates. As controls several kidney-sections from untreated mice were taken and handled for autoradiography as described above.

Column chromatography

Mice fed the vitamin D-deficient diet were injected intravenously with vitamin D dissolved in 25 μl abs. ethanol. One mouse received 0.1 μCi (4.8 μg) ^{3}H vitamin D_3 and one mouse received 0.1 μCi ^{3}H-vitamin D_3 together with 4.8 μg non-labelled vitamin D_3. 24 h later the animals were anaesthetized with ether The chest and the abdomen were opened, the vena cava was cut in front of the liver and the animals were perfused in the left ventricle with physiological saline, making the tissues almost empty of blood The kidneys were removed homogenized in 15 ml chloroform-methanol 2 1 (v/v) in a Potter Elvehjem glass homogenizer and extracted by the method of Bligh and Dyer (1959). Thus the homogenate was shaken overnight at 4 C. The chloro-

as was then separated after adding 2 volumes of water. The aqueous methanol residue was further [illegible] with 5 volumes of chloroform. The combined chloroform phases were evaporated to dryness [illegible] trogen and immediately redissolved in 0.2 ml n-hexane-chloroform 35:65 (v/v) to be used for chromatography. The column chromatography was performed at 4°C with Sephadex LH-20 [illegible] from Pharmacia Fine Chemicals, Uppsala, Sweden) (Holick and DeLuca 1971) using columns [illegible] equilibrated with n-hexane-chloroform 35:65 (v/v). The same solvent system was used for elution [illegible] fractions were collected. The fractions were evaporated with air to dryness in liquid scintillation [illegible] counted in a Packard liquid scintillation spectrometer after the addition of scintillation fluid [illegible] 0+0.25 g POPOP liter toluene). Correction for quenching was made by the use of an external [illegible] ^{3}H-vitamin D_3, ^{14}C-vitamin D_3 and ^{3}H 25-hydroxyvitamin D_3 were used as references to deter[illegible] elution volumes.

[illegible] counting

[illegible] accumulation of radioactivity in the kidney after the injection of ^{3}H-vitamin D_3 was determined [illegible] of mice fed the complete diet. Each mouse in the first series received 0.1 μCi (4.8 ng = 1.22 10^{-4} [illegible] ^{3}H-vitamin D_3 dissolved in 25 μl abs. ethanol. In the other series 0.1 μCi ^{3}H-vitamin D_3 was mixed [illegible] unlabelled vitamin D_3 and injected i.v. (dissolved in 25 μl ethanol). Thus in the second series each [illegible] received 48 ng (1.22 10^{-3} nmol) vitamin D_3, in the third series 0.48 μg = 1.22 nmol vitamin D_3, [illegible] fourth series 4.8 μg (12.2 10^{0} nmol) vitamin D_3 and in the fifth series 48 μg (1.22 10^{2} nmol) vitamin [illegible] [illegible] mice in each series were killed respectively 1 h, 2 h, 4 h, 8 h, 24 h and 48 h after the injection [illegible] vitamin D_3. They were perfused as described above (see under Column chromatography) and their [illegible] were removed. These were then dissolved in Soluene and the radioactivity was determined by [illegible] scintillation counting. With the knowledge of the specific activity of the parent vitamin the dis[illegible] per minute in the samples were converted to the absolute amount (pmol) of steroid in the [illegible].

[illegible] fractionation

[illegible] mice (fed the vitamin D-deficient diet) were injected i.c. with 0.1 μCi (4.8 ng) ^{3}H-vitamin D_3 (dis[illegible] in 25 μl dimethylsulfoxide). Six mice were killed at 4 h and six at 8 days after having first been [illegible]. 12 mice were injected in the same way with 0.1 μCi ^{3}H-vitamin D_3 together with 4.8 μg non-[illegible] vitamin D_3 and were killed at the same survival intervals as above (6 mice at each interval) after [illegible] been perfused. The kidneys were removed and manually homogenized in 10 ml 0.25 M sucrose in [illegible] glass tissue grinder with teflon pestle (Kontes Ltd. N.J. USA). The tissue suspensions were [illegible] through a double layer of gauze. Cellular fractionation was performed by centrifugation in 0.25 M [illegible] in M.S.E. high speed centrifuge (M.S.E. Ltd., London, England) first at 650 g for 10 min to [illegible] the nuclear fraction, then at 16 000 g for 20 min to obtain the mitochondrial fraction and finally [illegible] 5 000 g for 1 h to obtain the microsomal and supernatant fractions (r_{av} 109.7 mm). Microscopic ex[illegible] [illegible] of the nuclear fraction showed that it was practically free of contamination with unbroken cells [illegible] debris. The radioactivity of the fractions was determined by liquid scintillation counting after [illegible] in Soluene® as described above.

[illegible] an additional experiment two mice were injected i.c. with 0.1 μCi ^{3}H-vitamin D_3 together with 4.8 [illegible] unlabelled vitamin D_3 and were killed after 24 h. The kidneys were removed after perfusion and the [illegible] chondrial and microsomal fractions were isolated as described above. These fractions were then diluted [illegible] 5 ml sucrose and subjected to sonication in a Bronson Sonifier at 5A for 4 × 2 min (interruptions to [illegible] cooling) in an ice bath. The sonicated fractions were then centrifuged at 105 000 g for 1 h and the [illegible] activity in the resulting pellets and supernatants were determined by liquid scintillation counting as [illegible] described above.

Results

[illegible] body autoradiography

[illegible]. After the injection of ^{14}C-vitamin D_3 there was no difference in the distribution of [illegible] activity between the mice fed the complete diet and those which received the vitamin [illegible] deficient diet. At the studied survival intervals there was nor an obvious difference be[illegible] the i.v. and the s.c. injected animals. In the description below no distinction will [illegible] therefore be made between the different groups of animals.

Liver Kidney

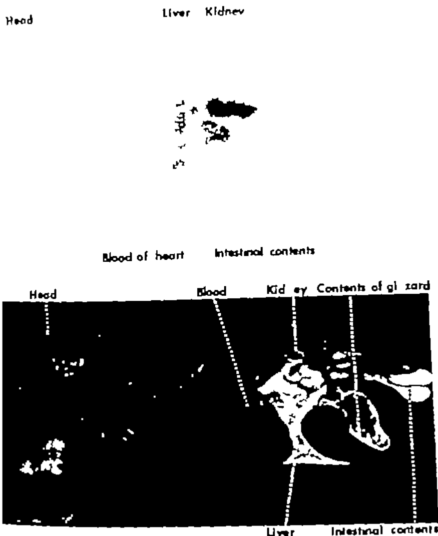
a

b

Liver Kidney

Fig. 1 *Autoradiographic distributio of vitamin D in the liver and the kidney* Details of hole autoradiograms of mice injected subcutaneously with 1 μCi (12 μg) ¹⁴C itamin D₃ a) 8 h and b) 18 d prior to death In) high accumulation of radioact vity (wh te areas) is present in the liver In the the concentration of radioactivity is rather low The cortex of the kidney shows a slightly higher ra activity than the rest of the kidney In b) the concentration of radioactivity in the li er is low but the kidney a considerable amount of radioactivity is present in the labyrinthic part of the cortex.

At short survival intervals (5 min–4 h) after the administration of labelled vitamin the radioactivity in the kidney was low in relation to organs such as the liver which accum lated a great amount of radioactivity At eight hours a specific distribution pattern appear in the renal cortex, but the radioactivity in the kidney was still lower than in the liver (F 1 a). 24 h after the injection of labelled vitamin D the radioactivity in the renal cortex w high, exceeding that of all other tissues in the body except the gastrointestinal content which contained a comparable amount of radioactivity (Fig. 2 a). At later survival interv the radioactivity in the kidney dominated the distribution pictures. Eighteen days after t injection a high concentration of radioactivity was still present in the renal cortex (Fig. 1 b



2. *Autoradiographic distribution of vitamin D_3. Comparison between mouse and quail.* Whole body radiograms of a) mouse and b) quail 24 h after subcutaneous injection of 0.5 µCi (4 µg) respectively (12 µg) ^{14}C-vitamin D_3. In the mouse high concentration of radioactivity (blue areas) is present in the renal cortex—a comparable concentration of radioactivity is found only in the intestinal contents. The concentration of radioactivity in the liver is considerably lower than in the kidney. In the quail the concentration of radioactivity in the kidney is not exceeding the level of the blood. The concentration of activity in the liver is considerably higher than in the kidney. The highest concentration of radioactivity was in the intestinal contents.

The radioactivity within the renal cortex was not evenly distributed but was concentrated in certain areas of the labyrinthic part. The extention of these areas indicated that they corresponded to the proximal tubuli. The remaining parts of the cortex, the medulla and the pelvis of the kidney had a low concentration of radioactivity. The same applied to the urinary bladder.



Fig. 3 *Microautoradiographic distribution of vitamin D_3 in the kidney* Renal specimens from a mouse injected with 3 μCi (36 μg) ^{14}C-vitamin D_3 24 h prior to death were used for the autoradiography. Frozen sections were taken in a cryostat at −15 C in a dark room directly on photographic plates. A glomerulus (G) with the connected proximal tubulus (P) is seen in the picture. Most silver grains are present in the proximal tubular cells. Htx.-eosin.G5 Ilford. (× 300).

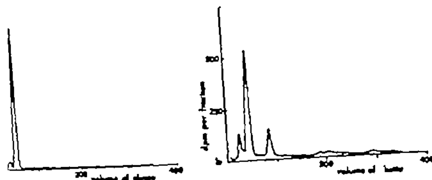
Quails. With respect to the kidney there was fundamental difference in the distribution patterns between quails and mice after the injection of ^{14}C vitamin D_3. Thus in the quail there was no specific accumulation of radioactivity in the kidney at any of the studied survival intervals (Fig. 2 b) The concentration of radioactivity never exceeded that of the blood and there was no specific uptake in any part of the nephron. The highest concentration of radioactivity in the quails was observed in the liver and the intestinal contents.

Microautoradiography

The microautoradiograms showed the silver grains to be specifically located over the cells of the proximal convoluted tubuli (Fig. 3). The labelling was found to begin at the junction between the glomeruli and the tubuli Specific labelling of sections from control mice was not observed

Column chromatography

The profiles of extracts from the kidneys chromatographed on Sephadex LH 20 are shown in Fig. 4 When a high dose (4 8 μg) of vitamin D was administered most of the radioactivity chromatographed in a position corresponding to that of vitamin D (Fig. 4 a). In addition one radioactive peak was found which had a lower elution volume than vitamin D_3. This peak probably represents an ester of vitamin D (see Discussion). When a low dose (4.8 ng) of vitamin D was administered most of the radioactivity still was found to chromatograph in a position corresponding to that of vitamin D (Fig. 4 b) One radioactive peak had a position corresponding to 25-hydroxyvitamin D_3. As at the high dose one radioactive peak was also found which had a lower elution volume than vitamin D_3.



[illegible]adiochromatographic profiles of kidney extracts after the administration of ^3H-vitamin D_3. [illegible] injected intravenously with ^3H-vitamin D_3 24 h prior to death, receiving 4.8 µg (Fig. 4) or (Fig. 4 b). The homogenized kidneys were extracted with chloroform-methanol and the chloroform was chromatographed on 1 × 80 cm Sephadex LH20 column. N-hexane-chloroform 35 : 65 (/v) [illegible] as elution medium. 5 ml fractions were collected.

[illegible] counting

[illegible]cribed above the autoradiograms showed that there was a slow uptake of radioactivity [illegible] kidneys upon the injection of labelled vitamin D_3. When this was studied by impulse [illegible]ting (Table I) it was shown that there was an increase in the radioactivity in the kidney [illegible] a maximal level 24 h after the injection of vitamin D_3. Still 48 h after the injection the [illegible]activity in the kidney was considerable, although lower than at the 24 h interval. [illegible] interesting to note that, at dose levels ranging from 4.8 ng to 4.8 µg of injected vitamin [illegible] the amount of steroid which was accumulated in the kidney was largely proportional [illegible] the given dose. At the highest dose level (48 µg) the accumulatory mechanism seemed to [illegible] been saturated, since the amount of steroid in the kidney was only about 2–4 times [illegible]her than the amount in the kidney at the closest dose-level (4.8 µg).

[illegible]ular fractionation

[illegible] results of the cellular fractionation showed that most of the radioactivity in the kidneys [illegible]er the administration of labelled vitamin D was found in the mitochondrial and the

TABLE I. Amount of steroid in the kidney at different survival intervals after the injection of vitamin D_3 at different dose levels. Each mouse received intravenously 0.1 µCi (4.8 × 1.22 10 nmol) ^3H-vitamin D_3 only or together with non-labelled vitamin D_3 in doses of 48 ng(1.22 10 nmol), 4.8 µg(1.22 nmol), 4.8 µg(1.22 10 nmol) and 48 µg(1.22 10 nmol). Three mice were used at each dose-level and survival interval. The radioactivity in their kidneys was determined. The disintegrations per minute in the samples were converted to the absolute amount (pmol) of steroid in the samples (mean ± S.E.).

Amount of injected vit. D_3 (nmol)	Amount of steroid in the kidney per 100 mg kidney-tissue (pmol)					
	1 h	2 h	4 h	8 h	24 h	48 h
$1.22 \cdot 10^{-4}$	$6.1 \pm 0.6 \cdot 10^{-4}$	$6.4 \pm 0.4 \cdot 10^{-4}$	$7.2 \pm 0.3 \cdot 10^{-4}$	$12.0 \pm 1.2 \cdot 10^{-4}$	$17.3 \pm 1.1 \cdot 10^{-4}$	$13.0 \pm 1.4 \cdot 10^{-4}$
$1.22 \cdot 10^{-1}$	$5.0 \pm 0.9 \cdot 10^{-}$	$6.4 \pm 0.7 \cdot 10^{-}$	$10.0 \pm 0.9 \cdot 10^{-}$	$15.5 \pm 1.0 \cdot 10^{-}$	$22.7 \pm 1.5 \cdot 10^{-1}$	$14.5 \pm 0.5 \cdot 10^{-}$
1.22	4.0 ± 0.6	5.2 ± 1.0	6.1 ± 0.9	$13.1 \pm$ 9	23.6 ± 3.6	15.0 ± 1.2
$1.22 \cdot 10^{1}$	$6.0 \pm 0.9 \cdot 10^{1}$	$9.6 \pm 0.7 \cdot 10^{1}$	$10.1 \pm 1.4 \cdot 10^{1}$	$14.5 \pm 0.6 \cdot 10^{1}$	$27.1 \pm 2.3 \cdot 10^{1}$	$12.1 \pm 0.1 \cdot 10^{1}$
$1.22 \cdot 10^{2}$	$4.2 \pm 0.4 \cdot 10^{2}$	—	$4.1 \pm 0.4 \cdot 10^{2}$	$5.6 \pm 0.9 \cdot 10^{2}$	$5.9 \pm 0.3 \cdot 10^{2}$	$3.4 \pm 0.2 \cdot 10^{2}$



Fig. 3 *Microautoradiographic distribution of vitamin D in the kidney* Renal specimens from a m injected with 3 μCi (36 μg) ^{14}C-vitamin D_3 24 h prior to death were used for the autoradiography Fro sections were taken in a cryostat at −15 C in a dark room directly on photographic plates. A glomer (G) with the connected proximal tubulus (P) is seen in the picture. Most silver grains are present proximal tubular cells. Htx.-eosin.G5, Ilford. (300).

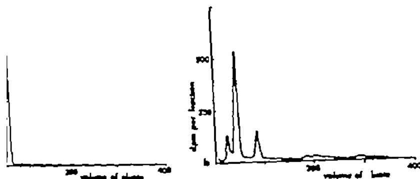
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[illegible]chromatographic profiles of kidney extracts after the administration of ^3H-vitamin D_3. [illegible] injected intravenously with ^3H-vitamin D_3 24 h prior to death, receiving 4.8 μg (Fig. 4) or [illegible] (Fig. 4 b). The homogenized kidneys were extracted with chloroform-methanol and the chloroform [illegible] was chromatographed on 1 × 80 cm Sephadex LH20 column. N-hexane-chloroform 35:65 ( ) [illegible] elution medium. 5 ml fractions were collected.

[illegible] counting

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[illegible] I. Amount of steroid in the kidney at different survival intervals after the injection of vitamin D_3 at different dose levels. Each mouse received intravenously 0.1 μCi (4.8 ng 1.22 10 nmol) ^3H-vitamin D_3 only or together with non-labelled vitamin D_3 in doses of 48 g(−1.22 10^{-4} nmol), 0.48 μg(1.22 nmol), 4.8 μg(−1.22 10^1 nmol) and 48 μg(−1.22 10^2 nmol). Three mice were used at each dose-level and survival interval. The radioactivity in their kidneys was determined. The disintegrations per minute in the samples were converted to the absolute amount (pmol) of steroid in the samples (mean ± S.E.)

[illegible] of [illegible] D_3 [illegible]	Amount of steroid in the kidneys per 100 mg kidney-tissue (pmol)					
	1 h	2 h	4 h	8 h	24 h	48 h
[illegible] 10^{-4}	6.1±0.6·10^{-4}	6.4±0.4 10^{-4}	7.2±0.3 10^{-4}	12.0±1.2 10^{-4}	17.3±1.1 10^{-4}	13.0±1.4 10^{-4}
[illegible] 10^{-4}	5.0±0.0·10^{-}	6.4±0.7 10^{-4}	10.0±0.9·10^{-4}	15.5±1.0·10^{-4}	22.7±1.5 10^{-}	14.5±0.5 10^{-}
[illegible]	4.0±0.6	5.2±1.0	6.1±0.5	13.1±2.9	28.6±3.6	15.0±1.2
[illegible] 10^1	6.3±0.9 10^1	9.6±0.7 10^1	10.1±1.4 10^1	14.5±0.6 10^1	27.1±2.3 10^1	12.1±0.1 10^1
[illegible] 10^2	4.2±0.4 10^2	—	4.1±0.4 10^2	3.6±0.9 10^2	5.9±0.3·10^2	3.4±0.2 10^2

TABLE II *Subcellular localization of radioactivity in the kidneys after the injection of labelled ...* Mice were injected s.c. with high (4.8 μg) and low (4.8 ng) doses of ^{3}H-vitamin D_3 ... after 24 hours and 8 days. Cellular fractionation was performed by centrifugation in ... sucrose. The radioactivity of the fractions was determined by liquid scintillation. The ... show the percent radioactivity in the respective fraction (mean ± S.E.).

	High dose (4.8 μg)		Low dose (4.8 ng)	
	4 h	8 days	24 h	8 days
Nuclei	13.2 ± 5.3	11.3 ± .9	12.9 ± 0.5	12.2 ± 2.2
Mitochondria	4..4 ± 9 6	45 6 ± 4.4	52.7 ± 1 6	36.9 ± 4 4
Microsomes	34.3 ± 4.5	27 8 ± 2.2	23.3 ± 0.8	39 7 ± 5.7
Supernatant	10.2 ± 0.3	15.3 ± 0.7	11 1 ± 0.2	11.. ± 1 6

microsomal fractions (Table II) Upon sonication of these fractions most radioactivi... found in the 105 000 g-pellet (97 4% of the radioactivity in the mitochondrial fr... 90.4% of the radioactivity in the microsomal fraction (mean of two experiments)). ... the radioactivity seems to be tightly bound to the mitochondrial and microsomal memb...

Discussion

The present application of whole body autoradiography to study the distribution of v... D has demonstrated the unique property of the proximal tubuli of the mouse kid... accumulated vitamin D_3. Eighteen days after the injection of labelled vitamin D a co... able amount of radioactivity was still present in the kidney It is interesting to note th... accumulation of vitamin D in the kidney takes place both in the mice receiving the vi... D-deficient diet and in those receiving the normal diet Vitamin D_3 has been repor... not being excreted in the urine (Cruickshank *et al* 1954). In our experiments a ve... radioactivity was found in the urine. In contrast to the situation in the mouse, the ... kidney was found to lack the ability to accumulate radioactivity above the level of the ... after the administration of labelled vitamin D_3. A greater radioactivity has been re... to occur in the kidney after the administration of labelled vitamin D in rats than in ... (Lawson et al 1971) Thus birds may lack a specific accumulatory mechanism for vi... D_3, present in the renal proximal tubuli of mammals. These differences may reflect imp... dissimilarities in the metabolism and/or physiological action of the vitamin, which s... be noted.

There was a slow increase in the amount of steroid in the kidney upon the intrav... injections of vitamin D_3, with a maximum being reached at 24 h Most of the vitam... was present in a nonmetabolized form. Similar results have been reported in the rat (No... and DeLuca 1963 Norman *et al* 1964 Lawson *et al* 1971). The reason for the slow u... in the kidney is not clear Metabolism of vitamin D prior to its accumulation in the k... obviously does not explain the delayed uptake The vitamin may enter the proximal tu... cells either via the tubular lumen, after having first been ultrafiltrated in the glom... or direct from the circulation, in the latter case by an accumulative process that ... slowly Vitamin D circulates in the plasma mainly attached to a transport protein (Th... *et al* 1959 Rikkers and DeLuca 1967 Peterson 1971 Edelstein *et al* 1973) Perhap...

has to occur before the accumulation in the kidney takes place. Far more experi- ed to clarify these points.

dney the proximal tubuli were found to accumulate the radioactivity. In dney the proximal tubuli were found to accumulate the radioactivity In ction of high dose of labelled vitamin D has also been shown to result in on of radioactivity in the proximal tubuli (Kodicek *et al.* 1961). The mito- the microsomes were found to be the organelles which accumulated most of ty Sonication of these organelles did not release the radioactivity A similar been reported in the rat (Norman and DeLuca 1964). These structures thus ve a large capacity for accumulating vitamin D_3, the amount being largely to the injected dose at several dose levels, as shown in the present study

itamin D_3 was present in the kidney mostly in a non-metabolized form after ration of both high and low doses of the vitamin, it was obvious that a change in so changed the chromatographic profile from the Sephadex column—so that vitamin D_3 was found in the kidney when a low dose of vitamin D had been he rat the liver seems to be the major site of 25-hydroxylation of vitamin D and DeLuca 1969). In vitro formation of 25-hydroxyvitamin D in the rat kidney reported as not occurring (Horsting 1970). The small peak which had a lower ists the vitamin D_3, probably represents an ester form of the vitamin. Vitamin have been found in the kidney and other tissues upon the administration of vitamin r and Kodicek 1968).

kidney is considered a site of action for itamin D_3, the parent vitamin as well as bolites are active in enhancing phosphate, sodium and calcium reabsorption (Gekle 71 Puschett *et al.* 1972 a, b). These processes take place principally in the proximal f the kidney (Durate and Watson 1967 Puschett *et al.* 1970), *i.e.* in the same areas accumulation of radioactivity occurs after the administration of labelled vitamin D ministration of high doses of vitamin D causes nephrocalcinosis most marked in oximal tubuli (*e.g.* Giacomelli *et al.* 1964). It is also interesting to note that vitamin iciency rickets may be associated with proximal renal tubular acidosis (Guignard and do 1973) and an increased secretion of amino acids via the proximal renal tubuli kle 1971). In the rat vitamin D-deficiency has been reported to result in swollen and thologically damaged kidney mitochondria (DeLuca *et al.* 1960). Vitamin D has been n to stimulate the release of calcium from isolated kidney mitochondria (DeLuca l. 1962).

References

...GH, E. G. and W. J. DYER, A rapid method of total lipid extraction and purification. *Canad. J. Biochem.* 1959 37 911–917

...BERG, L., Renale Aspekte der Rachitis. *Niederländisches Ärzteblatt* 1971 44 213–218.

...KOSCHMANN, E. M., E. KODICEK and P. ARMITAGE, The vitamin D content of tissues of rats given ergo- calciferol. *Biochem. J.* 1954 58 172–175.

DELUCA, H. F., ... RUBIN, H. STEENBOCK and P. KASHIMERO, Vitamin D and the structure of kidney mito- chondria. *Biochim. biophys. Acta* (Amst.) 1960 40 526–530.

DELUCA, H. F., G. W. ENGSTROM and H. RASMUSSEN, The action of vitamin D and parathyroid hormone ... on calcium uptake and release by kidney mitochondria. *Proc. nat. Acad. Sci.* (Wash.) 1962 48 1604–1609

DENCKER, L. and H TJÄLVE, Distribution of vitamin D : evidence of accumulation in renal prox tubuli and thyroid parafollicular cells. *Experientia* (Basel) 1973 *29* 719–722.

DUARTE, C. G and J F WATSON Calcium reabsorption in proximal tubuli of the dog nephron. *Amer Physiol.* 1967 *212*, 1355–1360.

EDELSTEIN S., D E. M LAWSON and E. KODICEK, The transporting proteins of cholecalciferol and hydroxycholecalciferol in serum of chicks and other species. *Biochem. J* 1973. *135* 417–426.

FRASER, D R. and E. KODICEK, Enzyme studies on the esterification of vitamin D in rat tissues. *Biochem.* 1968 *109* 457–467

GEKLE, D J STRÖDER and D ROSTOCK, The effect of vitamin D on renal inorganic phosphate reabsorption of normal rats, parathyroidectomized rats and rats with rickets. *Pediat Res.* 1971 *5* 40–52.

GIACOMELLI, F D SPIRO and J A. WIENER, Study of metastatic renal calcification at the cellular level *J cell Biol* 1964 *22* 189–206.

GUIGNARD J P and A TORRADO, Proximal tubular renal acidosis in vitamin D deficiency rickets. *Act paediat* (Uppsala) 1973 *62*, 543–546.

HOLICK M F and H F DELUCA, A new chromatographic system for vitamin D and its metabolites resolution of a new vitamin D metabolite. *J Lipid Res* 1971 *12*, 460–465.

HORSTING, M and H F DELUCA, In itro production of 25-hydroxycholecalciferol. *Biochem. biophys Res Commun.* 1969 *36* 251 256.

HORSTING, M *The nzymatic conversion f cholecalciferol to 25-hydroxycholecalciferol.* Ph D. Thesis University of Wisconsin Madison. 1970.

KODICEK E. E. M DARMADY and F STRANACK, The localization of [4]C-labelled vitamin D_2 in the nephron *Clin. Sci* 1961 *20* 185–195

KODICEK, E., The story of vitamin D f om vitamin to hormone. *Lancet* 1974. *1* 325–329

LAWSON E. E. M B. PELC P A. BELL, P W WILSON and E. KODICEK, Synthesis of (1,2-^{3}H$_2$)-cholecalciferol and metabolism of (4-^{14}C,1α-^{3}H)- and (4-^{14}C,1-^{3}H)-cholecalciferol in rachitic rats and chicks *Biochem. J* 1971 *121* 673–682.

NORMAN A W and H F DELUCA, The preparation of H^3 itamins D and D and their localization in the rat. *Biochemistry* 1963 *2* 1160–1168

NORMAN A W and H F DELUCA, The subcellular location of H -vitamin D_3 in kidney and intestine *Arch Biochem. Biophys* 1964 *107* 69–77

NORMAN A.W J Lund and H F DELUCA, Biologically active forms of tamin D_3 in kidney and intestine *Arch. Biochem.* 1964 *108* 12 21

NUMEROI P H L. SASSAMAN A ROGGERS and A E. SCHAEFER, The use of radioactive phosphorus in the assay of vitamin D *J Nut* 1955 *55* 13–21

PETERSON, P A., Isolation and partial characterizatio of a human vitami D-binding plasma protein *J biol Chem.* 1971 *246* 7748–7754

PUSCHETT J B Z. AGUS, D SENESKY and M GOLDBERG, Micropuncture study of the relationship of sodium to phosphate transport. *Clin. Res* 1970. *18* 700.

PUSCHETT J B., J MORANZ and W S KURNICK E idence for a direct action of cholecalciferol and 25-hydro ycholecalciferol on the renal transport f phosphate, sodium and calcium. *J lin. Invest* 1972 *51* 373–385

PUSCHETT J B P C. FERNANDEZ, I T BOYLE R W GRAY J L. OMDAHL nd H F DELUCA, The acute renal tubular effects of 1,25-dihydro ycholecalciferol *Proc Soc xp Biol* (N Y) 1972 b. *141* 379–384

RIKKERS, H and H F DELUCA, An in o study of the carrier proteins of H itamins D_3 and D_4 *Amer J Physiol* 1967 *213* 380–386.

STEENBOCK, H. and A. BLACK, F t-soluble tamins XVII The induction of growth-promoting calcifying properties in fats and their unsaponifiable constituents by exposure to light. *J biol Chem.* 1925 *64* 263–298.

THOMAS, W C. Jr H. G MORGAN, T B. CONNOR, L. HADDOCK C. E. BILLS nd J E. HOWARD, Studies of antirachitic activity in sera from patients w th disorders of calcium metabolism and preliminary observations on the mode of transport of tamin D human serum. *J lin. Inv st* 1959. *38*, 1078–1085

ULLBERG, S., Studies on the distribution and fat of S^{35}-labelled benzylpenicillin in the body *Acta radiol.* (Stockh.) Suppl. 1954. *118* 1 110.

ULLBERG, S., Autoradiographic studies on the distribution of labelled drugs in the body *Second U N Int Conf Peaceful Use of Atomic Energ.* 1958 *24* 248 254.

Acta physiol. scand. 1975. 95. 417–423
From the Department of Zoophysiology, University of Göteborg, Sweden

...stric Acid Secretion in a Teleostean Fish: A Method for the ...inuous Collection of Gastric Effluence from a Swimming Fish and its Response to Histamine and Pentagastrin

By

BJÖRN HOLSTEIN

Received 6 May 1975

Abstract

...IN, B. *Gastric acid secretion in a teleostean fish. A method for the continuous collec... ...n of gastric effluence from a swimming fish and its response to histamine and penta... ...strin.* Acta physiol. scand. 1975. 95. 417–423.

...acid secretion has been measured in the codfish (*Gadus morhua*) equipped with a catheter draining ...mach, by turning the water swallowed by the fish. Unstimulated acid secretion was found to be ...w in most fishes not exceeding 8 μmol H⁺/kg·h. Intramuscular injection of histamine evokes ...ependent secretion of gastric acid, with maximum acid output of 300 μmol/kg·h occurring with ...kg histamine dihydrochloride. No acid response was obtained with pentagastrin.

...ords: Fish physiology, gastric acid secretion, histamine, pentagastrin

...ertebrates possessing an acid secreting stomach that have been studied so far reveal ...secretion can be stimulated by exogenous histamine, except in the rays (Babkin *et al.* ...). Most of these studies have been carried out in the higher vertebrates, but few deals ...the lower vertebrates, *e.g.* fishes.

...teleosts, gastric acid secretion has been studied by Smit (1967). Acid secretion was ...ulated by distending the stomach with a piece of sponge, and the gastric juice was sampled ...taking out the sponge and squeezing it in distilled water. Grigoryan *et al.* (1968), working ...h the European catfish (*Silurus glanis*) showed that histamine elicited the secretion of an ...d gastric juice. The method used for the collection of gastric secretions was a modified ...ry-technique, with ligatures imposed upon the cardiac and pyloric parts of the stomach. The above workers employed sampling methods permitting only the collection of acid ...mulated in the stomach during a certain time. The present work describes a technique ...lowing the continuous collection of gastric effluent from unanaesthetized fishes and ...ents results showing that gastric acid secretion is dose-responsively stimulated by hista... ...ine, but is not affected by intramuscular administration of pentagastrin.

DENCKER, L. and H. TJÄLVE, Distribution of vitamin D_3 evidence of accumulation in renal tubuli and thyroid parafollicular cells. *Experientia* (Basel) 1973, *29* 719–72..
DUARTE, C. G and J F WATSON Calcium reabsorption in proximal tubuli of the dog nephron. *Physiol.* 1967 *212.* 1355–1360.
EDELSTEIN, S., D E. M LAWSON and E. KODICEK, The transporting proteins of cholecalciferol hydroxycholecalciferol i serum of chicks and other species. *Biochem J* 1973. *135.* 417–426.
FRASER, D R. and E. KODICEK Enzyme studies on the esterification of vitamin D in rat tissues. 1968 *109* 457–467
GEKLE, D J STRÖDER and D ROSTOCK, The effect of vitamin D on renal inorganic phosphate of normal rats, parathyroidectomized rats and rats with rickets. *Pediat Res.* 1971 *5.* 40–52.
GIACOMELLI F., D. SPIRO and J A. WIENER, Study of metastatic renal calcification at the cellular *J cell Biol* 1964 *22* 189–206.
GUIGNARD J P and A. TORRADO, Proximal tubular renal acidosis in vitamin D deficiency rickets *paediat* (Uppsala) 1973. *62* 543–546.
HOLICK M F and H F DELUCA, A new chromatographic system for vitamin D_3 and its metabolites resolution of a new vitamin D_3 metabolite. *J Lipid Res* 1971 *12* 460–465.
HORSTING, M and H. F DELUCA, In vitro production of 25-hydroxycholecalciferol. *Biochem. Biophys. Res. Commun.* 1969 *36* 251 256.
HORSTING, M *The enzymatic conversion of cholecalciferol to 25-hydroxycholecalciferol.* Ph.D. University of Wisconsin, Madison. 1970.
KODICEK E. E. M DARMADY and F STRANACK, The localization of [14]C-labelled vitamin D_2 in the ... *Clin. Sci.* 1961 *20* 185–195
KODICEK, E., The story of vitamin D from vitamin to hormone. *Lancet* 1974 *1* 325–329
LAWSON, E. E. M B. PELC P A. BELL, P W WILSON and E. KODICEK, Synthesis of (1- H)-calciferol and metabolism of (4- [14]C,1,2-[3]H$_2$)- and (4- C,1-[3]H)-cholecalciferol in rachitic rats and *Biochem. J* 1971 *121* 673–682.
NORMAN, A. W and H. F DELUCA, The preparation of H[3]-vitamins D_2 and D_3 and their localization the rat. *Biochemistry* 1963 *2* 1160–1168.
NORMAN, A. W and H F DELUCA, The subcellular location of H[3]-vitamin D_3 i kidney and ... *Arch. Biochem Biophys.* 1964, *107* 69–77
NORMAN, A. W J Lund and H F DELUCA, Biologically active forms of vitamin D_3 in kidney and ... *Arch. Biochem.* 1964. *108* 12 21
NUMEROF P H L. SASSAMAN, A. RODGERS and A E. SCHAEFER, The use of radioactive phosphorus the assay of vitamin D *J N* 1955 *55* 13–21
PETERSON, P A Isolation and partial characterization of a human vitamin D-binding plasma ... *J biol Chem.* 1971 *246* 7748–7754
PUSCHETT J B Z. AGUS, D SENESKY and M GOLDBERG, Micropuncture study of the relationship ... dium to phosphate transport. *Clin R* 1970. *18* 700.
PUSCHETT J B., J MORANZ and W B. KURNICK Evidence for a direct action of cholecalciferol and hydroxycholecalciferol on the renal transport of phosphate, sodium and calcium. *J clin. Invest.* *51* 373–385
PUSCHETT J B P C. FERNANDEZ, I T BOYLE, R W GRAY J L. OMDAHL and H F DELUCA. The renal tubular effects of 1,25-dihydroxycholecalciferol *Proc Soc ... Biol* (N.Y.) 1972 b. *141* 379-
RIKKERS, H and H F DELUCA, An in vivo study of the carrier proteins of H-vitamins D and D *J Physiol* 1967 *213* 380–386.
STEENBOCK, H. and A. BLACK, Fat-soluble vitamins XVII The induction of growth-promoting calcifying properties in fats and their unsaponifiable constituents by exposure to light. *J biol. Chem.* 1925 263–298.
THOMAS, W C. Jr H G MORGAN, T B CONNOR, L. HADDOCK C. E. BILLS and J E. HOWARD, Studies of antirachitic activity in sera from patients with disorders of calcium metabolism and preliminary observations on the mode of transport of vitamin D in human serum. *J clin. Invest.* 1959 *38.* 1078–10
ULLBERG, S., Studies on the distribution and fate of S[35] labelled benzylpenicillin in the body *Acta radiol.* (Stockh.) Suppl. 1954. *118* 1 110.
ULLBERG, S Autoradiographic studies on the distribution of labelled drugs in the body *Second U N Conf Peaceful Uses of Atomic Energy* 1958. *24* 248–254.

...ps and the final removal, is carried out by taking away the rubber stopper and the insertion finger into the oesophagus to guide the sond through the pharynx. The catheter was then ...ough the abdominal wall (X in Fig. 2) and the incision closed by continuous suture. ...the air trapped in the stomach during the operation water was injected through the catheter ...pe being opened by the straight (back) end of the sond. Injections of water was repeated until ...could be expelled.

...nd sampling. The operated fishes recovered from anaesthesia in aquaria with circulating water ...ere placed in tanks 10 × 35 cm in size. Water was continuously delivered to the tanks, water ...eld constant at 10 cm by letting the water drain through a trap, situated at 10 cm level on the ...The catheter was lengthened by connecting to another piece of tubing, passed through the trap ...ate fraction collector. The draining end of the catheter was placed about 10 cm below the ...which made the catheter work as a siphon.

...All injections were by the intramuscular route, and were either rapid (about 2 min) or slow ...tions. In the latter case motor-driven syringes were employed and the injection rate was 0.42 ...at 18 h elapsed between operation and the first injection.

...ation of acid content. The volume of each fraction was measured to the nearest 0.1 ml. 2-ml ...each fraction were then titrated with 0.01 M NaOH using phenolphthalein as an indicator. The ...water was titrated in the same manner, thus, giving the correction factor for the calculations of ...content of the water which had passed through the fish stomach. This method was used during ...he experiments reported in this paper. However the method was subsequently substituted by ...titrating using an autoburette (ABU 11), titrator (TTT 60) and PHM 61 pH-meter (Radiometer, ...gen), using 0.01 M NaOH. The end point was the pH of the aquarium water.

...ds. Histamine dihydrochloride (Sigma). Pentagastrin (Peptavlon, ICI) was kindly supplied by ...erole, Göteborg.

Results

...r output. The fishes usually recovered from anaesthesia in a few minutes. This was ...by the return of spontaneous ventilation and swimming. The drinking rate, however ...most fishes very low during the first 5–10 h postoperatively: the corresponding hourly ...ns sometimes contained less than one ml each, and were often empty. During the ...ing 2–4 h the drinking rate increased and remained higher though often variable. ...g the rest of the experiment most fishes delivered 4–10 ml/h, however great variations ...fishes were noted, and values of less than 1 ml/h and more than 40 ml/h were obtained. ...e efflux appeared in volleys of drops, with an interval of about 2 min between volleys.

...al acid secretion. Following the first 5–10 h after surgery when the fishes had resumed ...dy intake of water the acid output was very low in most fishes between 1 and 5 .../kg h. Sometimes no acid at all could be detected, i.e. the pH of the effluent water ...the same as the end point of the titration. The first fractions obtained from those fishes ...did not deliver any water during the first 5–10 postoperative hours, often contained a ...ter amount of acid, sometimes as much as 70 μmol/kg, when later they resumed drinking. ...accumulated acid was rapidly washed out and the output decreased to the 1–5 μmol/ ...h-level.

...he basal acid secretion value obtained (1–5 μmol/kg h) was before the drug administra-...to the fishes. In addition, several fishes were left unstimulated for 70–94 h, in 6 of these ...es the hourly acid output never exceeded 8 μmol/kg, but in two the acid production ...ed between 15 and 30 μmol/kg h.

Histamine. The rapid i.m. injection of histamine was followed by a dose-dependent ...ase of the acid content of the effluent water (Fig. 3, panel A). For the construction of the

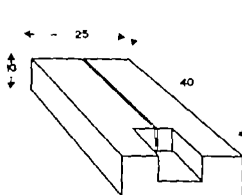


Fig. 1

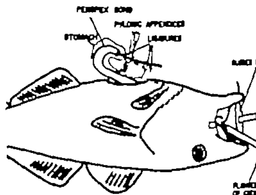


Fig. 2

Fig. 1 A clamp made from synthetic sponge was used to hold the fish during the operation. The clamped in the longitudal score (cut to 2/3 of the clamp s thickness) so that the head and gill-cov placed in the frontal outcut. Measures in cm.

Fig. 2. Implantation of the catheter in the stomach of the anesthetized codfish.

Material and Methods

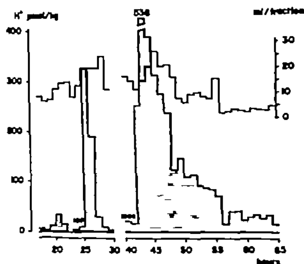
Animals. Codfishes (*Gadus morhua*), weighing 290–810 g of both sexes were used. Before the exp the fishes were kept in aquaria with circulating water at 10°C for at least one week. They are not

Surgical procedure Anaesthesia was induced by immersing the fishes in water containing 0 01 (Sandoz), and was complete when spontaneous ventilation ceased (2 5 min). The fishes were then tra to an operation table where respiration and anaesthesia were maintained by continuously floo gills (about 1 l/min) with water MS-222. The fishes were placed ventral side up and firmly held in by the clamp described below

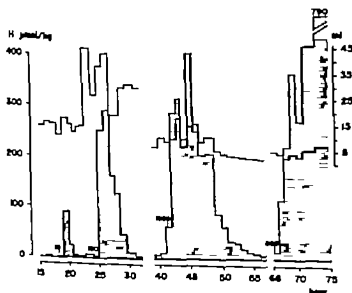
A convenient clamp for fish surgery was constructed from a piece of synthetic sponge rubber The clamp should be longer than the fishes and is cut in the mid-line to 2/3 of its thickness. A sm of the sponge is cut out at one end to accomodate the head and allow the gill-covers to open. Care be taken so that the gill-covers do not open in excess, so as to avoid only partial filling of the g with water By plugging in small pieces of sponges between the clamp and the gill-covers a suitable of the latter is easily achieved. Thus, the clamp can be adapted to accomodate fishes of differe Between operations, the clamp is stored in a well aerated aquarium as this keeps t soft and at the temperature.

The abdominal cavity was opened by a midline incision, about 3 cm long, between the pelvic fins anus. A perspex sond was then carried down the oesophagus nto the stomach. One end of the so bent to form a half circle (diameter 30 mm), and was taken down the oesophagus w th its bent end and pointing towards the right side of the fish. Then the sond was rotated 90° around its long a manoeuvred so that the opening of the bent end apposed the pyloric sphincter A catheter (Intra polyethylene tubing, PE-200) with a flanged back end, bout 50 mm longer than the sond as then through the perspex sond. The PE-200 tube was cut flat at the leading end. The catheter was gently through the pyloric valve and bout 20 mm into the ntestine where an incision I the intestinal w made, through which the tip of the catheter was taken out. 2 ligatures were then placed to prevent from the intestine (Fig. 2). By pulling the catheter through the sond the flange was placed close pyloric valve, it was prevented f m sliding int the testine by imposing third ligature just p to the pyloric appendices. Care was taken not to interrupt the blood flow in the main essels tr across that area.

To remove the sond, it is pressed in the posterio direction and t the same time, manoeuvred its bent end is lowered (.e moved dorsally). Duri g these movements, hich involves no rotation sond around its long axis, the muscular pyloric part of the stomach is pinched between the thumb index figer and pulled in the anterio-ventral direction The di tensibility of the tissues now ma withdrawal of the sond out of the curved stomach possible. At this stage the bent end of the soc



A



B

utput of acid and water in two fishes following different doses (μg/kg) of histamine dihydrochloride. dicate time elapsed since operation.

Discussion

idity of the present method is based on the assumption that the water swallowed by is not regurgitated after having mixed with the stomach content, but is quantitatively d through the catheter. No critical test was performed to prove that this is the case the arrangement of the catheter outlet secured an immediate delivery of the swallowed

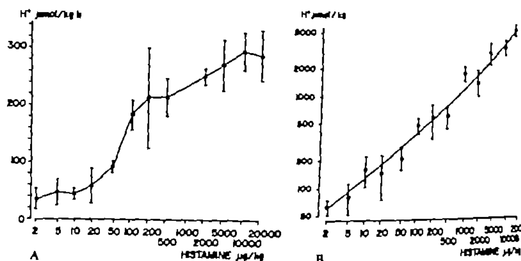


Fig. 3 Gastric acid secretion following graded doses of histamine dihydrochloride, given as rapid injections. Each fish was given 1 5 different doses. Values are means ±S.E. of 3–8 determinations. Responses were calculated as the mean of the two hourly fractions containing most acid. The resp following 1 000 µg/kg (362±53 n = 8) has been excluded from the graph. *B* Responses ere calculate the summed acid content of all fractions exceeding basal acid secretion.

dose-response curve, the values were calculated as the mean acid output of the two c secutive hourly fractions containing most acid. A delay in the appearence of this maxim with increasing doses of histamine was noticed for doses up to 500 µg/kg the peak appea within 3 h following injection, up to 5 mg/kg the peak was attained 2–6 h following injecti and for the highest doses as much as 10 h separated the injection from the peak output.

Fig. 4 shows examples of responses to different doses of histamine in two fishes. In addi to the increase of acid output there is also an increase in the duration of elevated production with increasing doses of histamine. Approximate values for the durations app in Table I The accumulated acid output, calculated as the sum of the acid content of fractions exceeding basal level, increased exponentially with increasing doses of histar (Fig. 3 panel B)

In two cases, following 10 mg/kg histamine, blood appeared in the effluent water

Pentagastrin. Pentagastrin was administered as rapid injections (25 50, 100, 250, µg/kg) and as slow injections during 5 h (50 100 200 400 µg/kg h). In no case could stimulation of gastric acid secretion be detected.

Since pentagastrin is synthetized with mammalian gastrin as a model, species differen could explain the ineffectiveness of the pentapeptide. To demonstrate endogenous gast codfish mucosae were extracted according to Blair *et al* (1961) and the product tested acid-stimulating power No acid response has been obtained (preliminary results).

TABLE I. Duration of elevated acid secretion following i.m. injection of histamine dihydrochloride.

Dose range	Duration
2 50 µg/kg	3–4
100–200 µg/kg	4–6
500–1 000 µg/kg	6–15
2–20 mg/kg	18–28

nean hourly output of acid following 5 mg/kg histamine amounts to
i the codfish.

s be concluded to be a powerful secretogogue in the codfish. Whether
ie is instrumental in stimulating gastric acid secretion in the mammals
itions is a matter of controversy the mechanisms in fish are totally
uirements enabling histamine to function as a final chemostimulator
tion in the codfish seem to be fulfilled, histamine is present in the gastric
able amounts (Reite 1969) and histamine powerfully stimulates gastric
ent investigation).

suggested to exert its acid-stimulating effect via the release of histamine
kson 1944), the secretion being sustained by an increased histamine-
i the mucosa triggered off by a feed-back mechanism involving the mucosal
thlson *et al.* 1964). According to a different opinion, the increased histidine
vity is mediated by gastrin (Johnson *et al.* 1969 Håkanson and Liedberg

lge, gastrin or pentagastrin has never before been tested in teleosts, like-
s of any report showing the presence of gastrin in fish. As neither penta-
fish mucosal extract was effective in stimulating gastric acid secretion it is
that the gastrin mechanism does not exist in the codfish. If this is correct
ic mucosa would be an interesting object for studying the correlations be-
d secretion and the activity of histidine decarboxylase.

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r Hakemer for the animal material.

References

F CHASSON and H. F FRIEDMAN, Factors determining the course of gastric secretion
in. *J Biol. Med. Chem.* 1933. *I* 251–259
A. HARPER, H. J LAKE, J D REED and T SCRATCHERD, A simple method of preparing
nal (Lond.) 1961 *156.* 11 P 12 P
O. KAHLSON, Histamine as physiological excitant of acid gastric secretion. *Acta physiol*
200–304.
M. G ZAKS and O. F TAMARYCHUK, Effect of histamine and pituitrin P on the gastric
e european catfish (*Silurus glanis* L.). *Problems Ichthyol.* 1968. *8.* 97–108. (Translation from
1968 *8.* 126–130.)
and G. LIEDBERG, Effect of pentagastrin and feeding on gastric histidine decarboxylase
i antrectomized rat. *Europ J Pharmacol.* 1972. *18.* 31–36.
L. ROSENGREN, D S. [illegible] and R. THOMSEN, Mobilization and formation of histamine in
ucosa as related to acid secretion. *J Physiol.* (Lond.) 1964. *174* 400–416.
V Effect of food swallowing on the intensity of digestive processes in fishes. *Vopr Ikhtiol.*
–137
Inhibition by acid of gastrin release. In: *Gastrin*, ed. M. I. GROSSMAN. UCLA Forum in
ences. No 5. University of California Press, 1966. 171–192.
ence of temperature on the rate of gastric juice secretion in the brown bullhead, *Ictalurus*
Comp. Biochem. Physiol. 1967 *21* 125–132.

, in Russian, is known to the author only as cited by SMIT H., Gastric secretion in lower
d birds. In: *Handbook of physiology* Sect. 6 Vol. V Ed. C. F CODE. American Physiological
hington, D.C. 1968. 2791 2805

water quantitative recovery seems probable. The finding of above basal levels of acid in first obtainable fractions from fishes which did not drink during a 5–10 h period follow operation also suggests that no leakage occurs through the oesophagus.

A comparison of volume and acid outputs (Fig. 4) reveals no correlation between th parameters. Often, an increase in acid output was accompanied by an increase in vol output or in some cases an increase in acid output was followed by an increased vol output However an increase in acid output may also occur simultaneously with a decr ing volume and *vice versa*. Furthermore, in the same fish, the coupling between acid volume outputs is variable from one instant to another (*i.e.* Fig. 4 panel B); following µg/kg histamine the increased acid output coincides with an increased volume out and the peak is delivered in a great volume of water In contrast to this is the far gre amount of acid delivered in about one sixth of the volume following 10 mg/kg histam The independence between acid and volume outputs further suggests that the metho reliable.

In most fishes, before drug injection, a slight acid production amounting to 1 5 µmol/1 could be detected. This basal acid secretion is 2–10 times as great as that reported by Gz *et al* (1968) for the European catfish. Besides species differences, there is one methodolo difference which might be relevant in explaining the discrepancy In mammals the relea gastrin from the antral mucosa is suppressed by a low antral pH (see Schofield 1966). gastrin mechanism is present in fish such suppression is more likely to take place u conditions where the acid accumulates in the stomach (method of Gzgzyan *et al.*) when the acid is continously washed away (present method).

Krayukhin (1959), working with the bullhead (*Ictalurus nebulosus*) found that swallo elicits an immense flow of gastric juice, and considers this to be evoked by an uncondit reflex. Perhaps the basal acid secretion in the codfish could be explained by such unc tioned reflexes, as it can be assumed that in the current experimental conditions, the f frequently swallow water In contrast to the results obtained by Krayukhin (1959) wit bullhead the act of swallowing *per se* does not seem to be a secretory stimulus of importance in the codfish.

The acid concentration following histamine administration is not reported in the pr investigation since this parameter is dependent on the volume of the water diluting the g juice. However to facilitate comparison with data obtained by other workers on g acid secretion in fish it might be pertinent to mention, that in low-volume samples (ab ml) acidities of 90 mM H have been encountered. After gastric distension the [H] for the bullhead was 249 mM at 25°C but at 10°C only 12 mM (Smit 1967). Gzgzyan (1968) obtained from the catfish, after 500 mg/kg histamine, a gastric juice with an a of 0.3 g% (82 mM). The dose may be regarded as a maximal one since following 1 mg/kg histamine the catfish produced a juice of about 40 mM [H].

The maximum rate of histamine induced gastric acid secretion in the codfish seems in the order of 300 µmol/kg h, occurring with 10 mg/kg histamine. Again, this is less the maximal rate of acid production in the bullhead (1 060 µmol/h, 25°C), but more the bullhead's production at 10 C–25 µmol/h (Smit 1967) Following 5 mg/kg histamin acid production in the catfish amounts to 4.5 µmol/h (Gzgzyan *et al* 1968), but the

r 24 h. The mean hourly output of acid following 5 mg/kg histamine amounts to 00 μmol/kg in the codfish.

mine can thus be concluded to be a powerful secretogogue in the codfish. Whether ous histamine is instrumental in stimulating gastric acid secretion in the mammals atural conditions is a matter of controversy the mechanisms in fish are totally n. Two requirements enabling histamine to function as a final chemostimulator tric acid secretion in the codfish seem to be fulfilled, histamine is present in the gastric a in considerable amounts (Reite 1969) and histamine powerfully stimulates gastric retion (present investigation).

ine has been suggested to exert its acid-stimulating effect in the release of histamine dm and Kahlson 1944), the secretion being sustained by an increased histamine-g capacity in the mucosa triggered off by a feed-back mechanism involving the mucosal ine level (Kahlson et al. 1964). According to a different opinion, the increased histidine oxylase activity is mediated by gastrin (Johnston et al. 1969 Håkanson and Liedberg

ny knowledge, gastrin or pentagastrin has never before been tested in teleosts, like-am unaware of any report showing the presence of gastrin in fish. As neither penta-n nor a codfish mucosal extract was effective in stimulating gastric acid secretion it is d to suggest that the gastrin mechanism does not exist in the codfish. If this is correct, odfish gastric mucosa would be an interesting object for studying the correlations be-n gastric acid secretion and the activity of histidine decarboxylase.

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References

m, B P A. F Crharon and H. F Friedman, Factors determining the course of gastric secretion duodenectomie. J. Biol. Med. Can. 1935. 1 251 259.

a, E. L., A. A. Harper, H. J Lake, J D Reed and T Scratcherd, A simple method of preparing stric J Physiol. (Lond.) 1961 156. 11 P 12 P

son, N and G. Kahlson, Histamine as physiological excitant of acid gastric secretion. Acta physiol. and. 1944. 8. 289–304.

ltan, D. M., M. G. Zaks and G. F Tanasiychuk, Effect of histamine and pituitrin P" on the gastric retion of the european catfish (Silurus glanis L.). Problems Ichthyol. 1968. 8. 97–100. (Translation from por. Ikhtiol. 1968. 8. 126–130)

Liedberg, R. and G. Liedberg, Effect of pentagastrin and feeding on gastric histidine decarboxylase ivity in the antrectomized rat. Europ. J Pharmacol. 1972. 18. 31 36.

lson, G., E. Rosengren, D. Svahn and R. Thunberg, Mobilization and formation of histamine in he gastric mucosa as related to acid secretion. J Physiol. (Lond.) 1964. 174 400–416.

seevich, B. V Effect of food swallowing on the intensity of digestive processes in fishes. Vopr. Ikhtiol. 959, 12 111–117

nstruct, B Inhibition by acid of gastrin release. In. Gastrin, ed. M. I Grossman. UCLA Forum in Medical Sciences. No 5. University of California Press, 1966. 171 192.

ett, B. Influence of temperature on the rate of gastric juice secretion in the brown bullhead, Ictalurus nebulosus. Comp. Biochem. Physiol. 1967 21. 125–132.

——

The article, in Russian, is known to the author only as cited by Smit H., Gastric secretion in lower vertebrates and birds. In: Handbook of physiology Sect. 6 Vol. V Ed. C F Code. American Physiological iety, Washington D.C. 1968

Acta physiol. scand. 1975. 95. 424–430

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On the Mechanism of Inhibitory Action of Vibrations as Studied in a Molluscan Catch Muscle and in Vertebrate Vascular Smooth Muscle

By

BENGT LJUNG and PER HALLGREN

Received 6 May 1975

Abstract

LJUNG B. and P HALLGREN *On the mechanism of inhibitory action of vibrations as in a molluscan catch muscle and in vertebrate vascular smooth muscle* Acta physiol. 1975 95 424–430

In previous studies longitudinal vibrations have been found to reduce active force development in muscle, possibly due to a direct action on the contractile mechanism. In the present experiments the tory effect of vibrations on isometric tension was studied in isolated preparations of the rat portal v rabbit thoracic aorta and the anterior byssus retractor muscle (ABRM) of the *Mytilus edulis*. Th demonstrate that vibrations of appropriate frequency and amplitude caused prompt inhibition of co tension and that complete recovery of act ve force normally occurred after cessation of vibration brate smooth muscle as well as during the phasic contraction of ABRM However in the "catch ABRM there was no regain in force following the vibration induced inhibition. The contractile pro considered to be in a locked stat during the catch situation. Thus, this contracted state seems to be by vibrations. It is therefore concluded that brations do nterfere with the interrelationship betw myofilaments. This conclusion supports the previously forwarded hypothesis that v brations ac creasing the rate of detachment of actin-myosin crosslinks in vertebrate smooth muscle.

Vibrational length changes have been found to reduce active isometric force exer vascular smooth muscle (Ljung and Sivertsson 1972, 1975) Similar inhibitory eff vibrations have previously been observed in striated muscle (*e.g* Mathews 1966, Rack and Westbury 1969) There is indirect experimental support that this inhibi caused by a mechanical interference with the contractile process in smooth muscle (and Sivertsson 1975) like in skeletal muscle, where an increased rate of actin-myosin cro detachment has been suggested to occur (Joyce *et al* 1969)

In some molluscan muscles, such as the anterior byssus retractor muscle (ABRM) *Mytilus edulis* two different types of contractile responses can be elicited when either means of stimulation are employed or environmental conditions are altered (see Low Millman 1963 Twarog 1967). The distinction is apparent from the rate of tension after activation. Stimulation with alternating current, repetitive electrical impulses or

-choline (Ach) at high temperatures produces a response which rapidly declines. Where-as direct current activation or Ach at "sea" temperature results in a contraction which only slowly decays after stimulation. In the latter type of response two phases can be detected. The first component represents a tetanic contraction functionally similar to responses in vertebrate smooth muscle. It is followed by a "catch" phase where the induced tension is maintained at a low metabolic rate (see Lowy and Millman 1963). Administration of 5-hydroxytryptamine (5-HT) to the muscle in catch phase leads to rapid relaxation. It is a matter of debate as to the exact mechanism by which the muscle maintains tension in the catch state (for ref. see Rüegg 1971). However there seems to be little doubt that the catch mechanism can be attributed to some process which prevents the sliding of adjacent contractile filaments relative to each other.

It appeared that a comparison of the influence of vibrations on active force in vertebrate smooth muscle and on the phasic and catch components of lamellibranch smooth muscle responses would indicate whether vibrations inhibit active force by an action on the contractile mechanism. In the present experiments isolated preparations of the rat portal vein, the rabbit thoracic aorta and the ABRM were exposed to longitudinal vibrations.

Methods

The method for studying the effects of vibrations on the vascular smooth muscle of the rat portal vein and the spiral strip of the rabbit thoracic aorta has recently been described in detail (Ljung and Sivertsson 1975). In summary, the vessels were carefully dissected after the animals had been sacrificed. The smooth muscle preparations, approximately 10 mm in length, were mounted in the longitudinal direction between a force transducer (Grass FT .03) and a vibrator. The latter was fed sine wave current to impose length changes, ± 100 μm in amplitude at a frequency of 100 Hz. A passive force of 5 and 15 mN was applied to the portal vein and the aortic spiral strip preparations, respectively.

The tissues were allowed to accommodate for one h before the experiment was started. The Krebs solution used for the vascular tissue had the following composition in mM: NaCl 122, KCl 4.73, $NaHCO_3$ 15.5; KH_2PO_4 1.19, $CaCl_2$ 2.40 and Glucose 11.5. It was continuously bubbled with 4 % CO_2 in O_2 and kept at 37°C.

The anterior byssus retractor muscle (ABRM) was obtained from fresh mussels (*Mytilus edulis*). The shell was opened by cutting the adductor muscles. One of the retractor muscles was cut in order not to stretch the other one. After removal of adjacent tissue the remaining retractor muscle was carefully split longitudinally into two halves. One half was firmly tied *in situ* with surgical silk to provide a 15 mm long preparation, which was attached to the force transducer (Grass FT .03 with rod & black springs) at one end and to the vibrator at the other end. The muscle was stretched to 70 % of its *in situ* length in the presence of 5-HT (10^{-6} M). The organ bath contained filtered seawater (690 mOsm), continuously bubbled with 4 per cent CO_2 in O_2. The temperature was normally kept at 8–12°C. The muscle was allowed to accommodate one h before the actual experiment began.

The following drugs were used: *5-hydroxytryptamine* (FLUKA AG), *acetylcholine hydrochloride* (E. MERCK AG) and noradrenaline bitartrate (l-arterenol, SIGMA).

Results

Mammalian vascular muscle. Tracings from expts. on the rat portal vein preparation (A) and the rabbit thoracic aorta (B) are shown in Fig. 1. The spontaneous activity of the isolated rat portal vein preparation (A) consisted of phasic contractions occurring at a frequency of 2–4 per min with intervening periods of inactivity where the baseline level of passive force

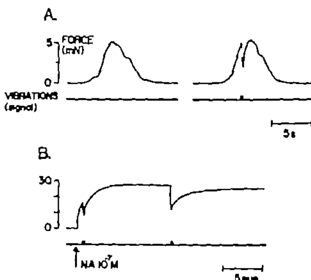


Fig. 1 Tracings of spontaneous contracti of the rat portal vein (A) and of a noradrena line (NA) induced response of the rab aorta (B). Vibrations applied at signal. No rapid decrease in force caused by vibrati and regain in active force after the vibra periods.

prevailed. The left recording in Fig. 1 A represents one such spontaneous contractio Mechanical vibrations (100 Hz, ± 100 μm) were applied during the period of time indicat by the signal in the right recording of panel A. It is seen that the vibrations caused a promp decrease in active force, followed by a somewhat slower regain after the vibration perio Apart from this short lasting, vibration-induced interference with active force the configur tion of the spontaneous contraction was not altered.

The NA response of the rabbit thoracic aorta (Fig. 1 B) was characterized by a smoo increase in force until a stable level of activity was reached. In the expt. illustrated in Fig. 1 vibrations were applied during the rising phase of the response to NA (10⁻ M) and duri the response plateau 10 min later. In both of these cases vibrations caused immediate redu tions of active force of the aortic smooth muscle followed by a gradual recovery after te mination of the oscillations. Thus, the imposed length changes induced reversible inhibito responses in vascular smooth muscle. The rate of the recovery after cessation of vibratio was greater in the portal vein than in the aortic preparation.

Molluscan catch muscle. The contractile response of the ABRM to acetylcholine (Ac at 12°C, is illustrated in the top recording of the left panel of Fig. 2. Typically a peak for level was reached during the first min after administration of Ach and thereafter for receded gradually. The relaxation occurred at the same low rate whether or not the agon was rinsed out after 4 min exposure. Vibrations applied during the rising phase of t Ach response (middle and lower recording) caused a transient reduction of tension. contrast, only little regain of force was seen when vibration-induced inhibition was elicit either 2.5 min after the administration of Ach, *i.e.* before rinsing, or after 5 min *i.e.* after A had been removed (middle and lower tracings, respectively of Fig. 2, left panel). The rig panel of Fig. 2 illustrates responses to Ach of the ABRM in sea-water at 37°C. The cont response (upper tracing) was characterized by a transient peak contraction which oft displayed a phasic component. Vibrations applied 30 s after onset of the contraction result in an abrupt, short lasting inhibition. Occasionally a second phasic contraction was a parently elicited by vibrations (lower tracing). Thus, the imposed oscillations caused t

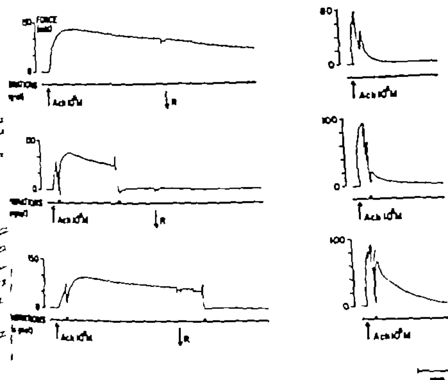


Fig. 2. Responses of the ABRM to acetylcholine (Ach) in sea-water at 12°C (left panel) and at 37°C (right panel). Upper recordings show control responses to the agonist, which was rinsed out at R. Middle and lower recordings show effect of vibrations applied at signal. Note lack of force recovery after the second bursts of vibration applied at 12°C (left panel, middle and lower recordings).

same pattern of reversible inhibition of the phasic response at 37°C as it did during the rising phase at 12°C. This is in contrast to the non-reversible inhibition of the ABRM obtained at 12°C 2.5 or 5 min after the induction of the response.

The tracings of Fig. 3 taken from one expt. on the ABRM with repeated exposures to Ach, illustrate the relationship between time of exposure to Ach and effects of vibrations. Brief oscillations applied after 5 and 10 min of Ach exposure (top and second tracings, respectively) caused complete and sustained abolition of active force. Succeeding applications of vibrations at decreasing time intervals after the Ach injection were followed by increasingly more pronounced recoveries, so that the contraction amplitude which ensued vibrations applied 15 s after Ach injection (bottom recording) was actually greater than the initial peak response level. The sequence of effects of vibrations in Fig. 3 thus demonstrates that the degree of maintained inhibition following the oscillations depends on the time elapsed after the administration of the agonist.

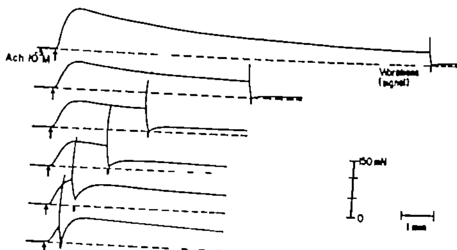


Fig. 3 Effect of vibrations on ABRM responses to acetylcholine (Ach). Vibrations applied at dif periods after administration of the agonist. Note that increasingly greater recoveries occurred tervals decreased between onset of response and application of vibrations.

The same sequence of vibrations, applied during a single period of Ach exp illustrated in Fig. 4 The control response to Ach is shown by the contour of the shac After repeated rinses and complete muscle recovery Ach was again administered an tions were applied after 0.3 0.6, 1.25 2.5 5 and 10 min. It is seen from the two s posed recordings that during the rising phase of the response, pronounced inhibit induced followed by complete recovery From the peak response level and on, succ less regain of force was seen after vibrations and 5 min after Ach injection hardly an tension was reestablished

Discussion

Vibrations were found to cause prompt reductions of active force when applied i experiments. Complete regain of tension gradually followed the brief vibration p

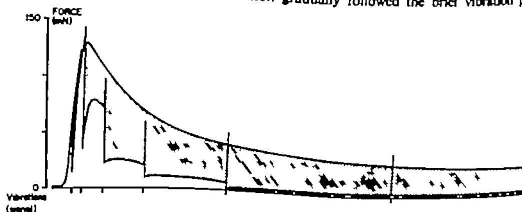


Fig. 4 Effects of vibrations on ABRM response to acetylcholine (10^{-4} M). Experiment recorded on and replayed on rectilinear recorder The pper contour of the shaded area represents control resp and that of the open area shows a superimposed recording of a subsequent response here vibrations repeatedly applied at signal. Note that complete recovery of the contraction occurred after the initial not after subsequent vibrations.

a vertebrate smooth muscle and under certain conditions in the ABRM. In contrast, y any recovery as seen when vibrations were applied a few minutes after the onset of nction in experiments at a temperature of 12°C. Thus, there is a clear distinction be- a the reversible inhibition found in vertebrate smooth muscle, in the ABRM at 37°C a the early response of the ABRM on the one hand and the maintained inhibition during ate stage of the ABRM response at 12°C on the other hand.

e particular properties of some molluscan muscles, like the ABRM have attracted h interest. It is known that stimulation with either alternating current (Winton 1937) or tive impulses (Lowy and Millman 1963), or with exogenous Ach at a temperature a 30°C (T uog 1967) leads to a "phasic contraction which quickly decays. If instead ABRM is exposed to direct current or Ach at low temperatures, the initial "phasic" one is followed by a maintained tension associated with low energy consumption h gives way very slowly. Since the ABRM appears to be caught, this contraction phase been called the catch of the muscle. It seems clear that the maintained inhibition obtained e ABRM after vibration reflects release of the catch mechanism. Thus, the progressive lution of regained force found with increasing interval between onset of response and lication of vibrations (Fig. 3) is explained by the time course of the phasic response ponent. Repeated exposures to vibrations *during one single response* (Fig. 4) would separate the contribution to the force development provided by the active and the catch hanism, respectively. Two main explanations have been given for the catch mechanism. rding to the paramyosin hypothesis the sliding of adjacent contractile protein fila- ts is hindered by shearing forces applied by the paramyosin protein, which is abundantly nd in catch muscles (see Ruegg 1971). The "linkage hypothesis forwarded by Lowy lman and Hanson (1964) suggests that the crosslinks between actin and myosin fila- nts, cyclicly formed and detached during the early phase of contraction, only detach ery ly during the catch phase.

The present experiments do not seem to offer conclusive support for either of the two lanations of catch. However they do demonstrate that vibrations abolish the catch ten- n. This effect must be accomplished by a mechanical separation of the caught contractile ments. It is reasonable to conclude that the reversible inhibition of the phasic ABRM traction likewise is due to interference with the contractile process *i.e.* detachment of mplementary bindings.

By analogy the present results strongly support the hypothesis (Ljung and Sivertsson 1975) t the pronounced inhibitory effects of oscillating length changes in vertebrate smooth scle is due to increased rate of detachment of crosslinks between the contractile filaments.

pported by the Swedish Medical Research Council (3484). Thanks are due to Miss Ann Kjellstedt for technical assistance and to Miss Lena Nonnerfors for secretarial aid.

References

rtel, G. C., P. M. Rack and D. R. Westbury. The mechanical properties of cat soleus muscle during controlled lengthening and shortening movements. *J. Physiol.* (Lond.) 1969. 204. 461–474.
ng, B. and R. Sivertsson. The inhibitory effect of vibrations on tension development in vascular smooth muscle. *Acta physiol. scand.* 1972. 85. 428–430.

LJUNG, B. and R. SIVERTSSON, Vibration-induced inhibition of vascular smooth muscle contraction. *Blood vessels.* 1975 *12*, 38–52.

LOWY J and B. M MILLMAN, The contractile mechanism of the anterior byssus retractor muscle of *My. edulis. Philos. Trans.* 1963 B. *246*, 105–148.

LOWY J., B. M. MILLMAN and J HANSON, Structure and function in smooth tonic muscles of lamellibr molluscs. *Proc. roy Soc B* 1964 *160* 525–536.

MATTHEWS, P B. C., The reflex excitation of the soleus muscle of the decerebrate cat caused by vibr to its tendon. *J Physiol* (Lond.) 1966. *184* 450–472.

RÜEGG, J R. Smooth muscle tone. *Physiol. Rev* 1971 *51* 201–248.

TWAROG, B. M. Factors influencing contraction and catch in *Mytilus* smooth muscle. *J Physiol.* (L 1967 *192*, 847–856.

WINTON, F R., The changes in viscosity of an unstriated muscle *Mytilus edulis* during and after stimul with alternating, interrupted and uninterrupted direct currents. *J Physiol.* (Lond.) 1937 *88*, 49

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Rubrospinal Control of Static and Dynamic Fusimotor Neurones

By

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Abstract

…berg, B., T. Jeneskog and H. Johansson. *Rubrospinal control of static and dynamic fusimotor neurones.* Acta physiol. scand. 1975. 95 431–440.

…spinal effects on about 60 extracellularly recorded γ-motoneurones were studied in anaesthetized cats. …ls were functionally identified from … known muscle nerves. 23 cells were regarded as dynamic as … were activated from … mesencephalic region previously known to influence selectively muscle spindle …mic sensitivity. The pattern of rubrospinal influence on static fusimotor neurones to different muscles …y followed that previously demonstrated for α-motoneurones with predominantly excitation of …r neurones and excitation or inhibition in equal amounts of extensor cells. Dynamic fusimotor neu… …were influenced in … strictly reciprocal manner with excitation of flexor cells and inhibition of ex… …r cells except for … *few neurones* …hich could not be reached from nucleus ruber. Evidence was also …ined indicating that *the shortest path from nucleus ruber to static fusimotor neurones involves one* …neurone.

…e effect of *electrical stimulation within the red nucleus on the fusimotor system was* …ied by Appelberg (1962) and by Appelberg and Kosary (1963) by the indirect method …recording from muscle spindle afferents. It was regularly observed that such stimulation …sed inhibition of spontaneous activity in extensor spindles while flexor spindles were …ead excited. These findings were in general agreement with the notion at that time that …e red nucleus was excitatory to flexor and inhibitory to extensor muscles (Sasaki, Nami…ra and Hashikamoto 1960).

…e recent years the control function of the rubrospinal tract as well as of other systems …cending from the mesencephalon and acting on the neuronal machinery in lumbar seg…ments have been extensively and carefully studied by Lundberg and coworkers (*cf* Hongo, …ankowska and Lundberg 1969 *a, b* 1972 *a, b and Baldissera, Lundberg and Udo* 1972 *a, b*). …hese studies have, among other things, given extremely detailed information about rubro…spinal control of hind limb α-motoneurones and also carefully analyzed methodological …spects of importance when using electrical stimulation in the rubral region.

…With this in mind and considering current theories of linkage between α- and γ-moto…neurones in motor control it seemed of great interest to reinvestigate in greater detail and

LJUNG, B. and R. SIVERTSSON, Vibration-induced inhibition of vascular smooth muscle contraction. *Blood vessels.* 1975. *12.* 38–52.

LOWY J and B. M MILLMAN The contractile mechanism of the anterior byssus retractor muscle of *M edulis. Philos. Trans.* 1963. B. *246.* 105–148

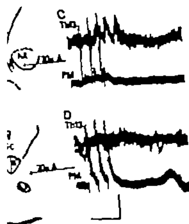
LOWY J., B. M. MILLMAN and J HANSON Structure and function in smooth tonic muscles of lamellib molluscs. *Proc roy Soc B* 1964 *160* 525–536.

MATTHEWS, P B. C., The reflex excitation of the soleus muscle of the decerebrate cat caused by vi to its tendon. *J Physiol.* (Lond.) 1966. *184.* 450–472.

RÜEGG, J R., Smooth muscle tone. *Physiol. Rev.* 1971 *51* 201 248.

TWAROG, B. M Factors influencing contraction and catch in *Mytilus* smooth muscle. *J Physiol.* (L 1967 *192.* 847–856.

WINTON, F R., The changes in viscosity of an unstriated muscle *Mytilus edulis* during and after stim with alternating, interrupted and uninterrupted direct currents. *J Physiol.* (Lond.) 1937 *88.* 49



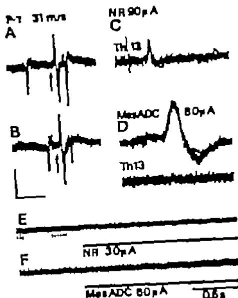


Fig. 1 Fig. 2

Fig. 1. A and B are camera lucida drawings of transverse sections through the mesencephalon at rostral (B about 1 mm caudal to A) with stimulating sites indicated. Three stimulating shocks at 600 Hz to site in A activates rubrospinal tract as revealed by surface recording from the cord in C (upper trace). The stimulating point in B causes no rubrospinal volley (D, upper trace) but instead climbing fibre response in the paramedian lobule of the cerebellum is evoked (D lower trace). Positivity is recorded upwards in this and all subsequent figures. Time calibration 5 ms, voltage calibration 100 μV to upper traces in C and D, 200 μV to lower traces.

Fig. 2. A, B, identification of plantaris fusimotor neurone (see text). C, descending volley in Th_{13} to NR stimulation. D PM-response evoked from MesADC (upper trace). Note lack of descending volley (lower trace). Time calibration below B is 10 ms to A, B and D, 4 ms to C. Voltage calibration is 100 μV for upper trace in D. E, inhibition of spontaneous fusimotor activity by repetitive stimulation in NR during time indicated by black line below record. F activation of same fusimotor neurone from MesADC.

An equally selective activation of the descending pathway to dynamic fusimotor neurones was attempted by placing a second stimulating electrode dorsally in the low-threshold region for D-zone climbing fibre response in the PM. A direct activation of rubrospinal cells could usually be obtained from this region, but only at high stimulating intensities. Only effects on γ-motoneurones at strengths well below the threshold for NR-activation were considered. Also at such lower stimulating strengths a descending volley could sometimes be recorded in the thoracic segment during train stimulation. This volley was similar to that described by Baldissera *et al.* (1972 a) as being characteristic for the dorsal reticulo-spinal system. In Fig. 1 the position of stimulating sites for rubro-spinal activation (A) and within the MesADC (B) in one experiment, where histological control was made, is illustrated. In C and D the physiological criteria used for correct placement in all experiments are illustrated. In a few early experiments only NR-stimulation took place and of the total cell-material of 63 cells 13 were thus not tested from MesADC (see under Results).

Results

1. *Effects from NR and MesADC on γ-motoneurones*

Table 1 summarizes the results obtained in one experiment (same experiment as Fig. 1). N... motoneurones were recorded from, 6 of which were completely tested with regard...

with another technique the matter of rubrospinal control of hind limb fusimotor neuro A detailed knowledge of a descending system selectively influencing dynamic fusimo neurones (*cf* Appelberg and Jeneskog 1972, Jeneskog 1974 a) was utilized as a methodolo cal tool allowing the separation of γ-motoneurones into the two functional groups, static a dynamic.

Methods

The results to be presented were obtained in experiments on 18 cats. Of these 11 ere anesthetized halothane (usually 1.0–1.5 %) administered in a mixture of Oxygen (1/3) and Nitrous Oxide (2/3), 4 α-Chloralose (60 mg/kg) administered intra enously after induction with halothane and 3 th pe barbital (40 mg/kg) given intraperitoneally w th later supplementary intra enous doses. Essentially sim findings were obtained regardless of type of anesthesia. Blood pressure and expiratory CO_2 as well as re temperature and temperatures in the paraffin pools were continuously monitored throughout the periments.

Operation

The operative procedure included a low thoracic and a lumbar laminectomy The dorsal columns and t right spinal half were sectioned in Th12 13 The lumbar spinal cord was prepared for microelectr recording from hind limb motoneurone pools.

In the left hind limb the following nerves were dissected and mounted for stimulation posterior bice semitendinosus (PBSt), anterior biceps-semimembranosus (ABSm), gastrocnemius-soleus (GS), planta (P), flexor digitorum and hallucis longus (FDL), deep peroneal (DP), superficial peroneal (SP) and tib (Tib). In one experiment the tenuissimus nerve was prepared instead and later used for dissection of f ments (see Results, Fig. 3).

The sensorimotor cortex was acutely ablated bilaterally in all experiments.

Access to the right mesencephalon for stimulating electrodes and to the left paramedian lobule of t cerebellum for surface recording was arranged.

Recording

Extracellular recording was made from γ-motoneurones with glass capillary electrodes filled with 4 NaCl. The impedance of the electrodes was usually bout 1 2 MΩ. Only cells which could be antidromica invaded from one of the dissected muscle nerves and thus identified were accepted. The conduction velo ties of the axons could then be determined by also measuring the conduction distance along the different nerves after each experiment Only cells ha ing a spontaneous activity could be tested with regard to i hibitory influence from the brain, and therefore each cell included in the material exhibited such ac tivity during at least part of the recording session.

Surface recording of the rubrospinal tract volley on the dorsolateral aspect of the cord in the expo thoracic segment was employed to guide the placement of a stimulating electrode n the red nucleus (NR

Surface recording of climbing f bre responses in the D-zone of the cerebellar paramedian lobule (PM was employed when placing a stimulating electrode in a mesencephalic area previously show to selectiv influence dynamic fusimotor neurones (MesADC —cf Appelberg 1967 and Jeneskog 1974 a and Discu sion).

Stimulation

For stimulation in the brain tw medio-lateral grids of 3 glasscovered platin m-iridium electrodes w 1 mm interelectrode distance were used. One of the grids was angled 15 to the ertical plane as all ele trodes had to be positioned at approximately the same rostro-caudal level but at different depths.

A presumably selective activation of the rubro-spinal tract was obtained by stimulation of interposit rubral fibres in the caudo-ventral part of or just ventrally to the caudal part of the red nucleus. The corre placement of the stimulating lectrode was aided by bserv g the descending olley change from a shor latency direct response to transsynaptic response w th longer latency when slowly proceeding th the electrode through the red nucleus (f Baldissera *et al* 1972 b). This placement allowed maximal activa tion of the rubro-spinal tract t low stimulating strength t a considerable distance away from the MesADC,

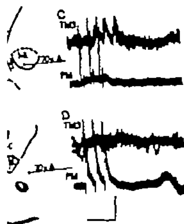


Fig. 1

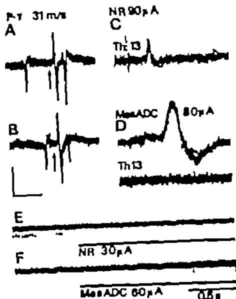


Fig. 2

A and B are camera lucida drawings of transverse sections through the mesencephalon at rubral (B about 1 mm caudal to A) with stimulating sites indicated. Three stimulating shocks at 600 Hz to the site in A activates rubrospinal tract as revealed by surface recording from the cord in C (upper trace). The stimulating point in B causes no rubrospinal volley (D, upper trace) but instead a climbing fibre response in the paramedian lobule of the cerebellum is evoked (D, lower trace). Positivity is recorded upwards in this and all subsequent figures. Time calibration 5 ms, voltage calibration 100 µV to upper traces in C and D, 200 µV to lower traces.

Fig. 2. A, B, identification of phasic fusimotor neurones (see text). C, descending volley at Th_{13} to NR stimulation. D, PM-response evoked from MesADC (upper trace). Note lack of descending volley (lower trace). Time calibration below B is 10 ms to A, B and D, 4 ms to C. Voltage calibration is 100 µV for lower trace in D. E, inhibition of spontaneous fusimotor activity by repetitive stimulation in NR during period indicated by black line below record. F, activation of same fusimotor neurone from MesADC.

The equally selective activation of the descending pathway to dynamic fusimotor neurones was attempted by placing a second stimulating electrode dorsally in the low-threshold region for the D-zone climbing fibre response at the PM. A direct activation of rubrospinal cells could usually be obtained from this region, but only at high stimulating intensities. Only effects on γ-motoneurones at strengths well below the threshold for NR-activation are considered. Also at such lower stimulating strengths a descending volley could sometimes be recorded in the thoracic segment during train stimulation. This volley was similar to that described by Baldissera et al. (1972 a) as being characteristic for the dorsal reticulo-spinal system. In Fig. 1 the position of stimulating sites for rubro-spinal activation (A) and within the MesADC (B) in one experiment, where histological control was made, is illustrated. In C and D the physiological criteria used for correct placement in all experiments are illustrated. In a few early experiments only NR-stimulation took place and of the total cell-material of 63 cells 13 were thus not tested from MesADC (see under Methods).

Results

1. *Effects from NR and MesADC on γ-motoneurones*

Table 1 summarizes the results obtained in one experiment (same experiment as Fig. 1). Nine γ-motoneurones were recorded from, 6 of which were completely tested with regard to

TABLE I Effects from nucleus ruber (NR) and mesencephalic area for dynamic control of muscle (MesADC) on nine fusimotor neurones in 1 expt. Thresholds for effects given in μA. + tion, − = inhibition 0 = no influence.

Cell no.	Type	Cond. vel.	NR	μA	MesADC	μA
1	FDL	32 m/s	−	50	+	60
2	DP	32 m/s	+	50	0	70
3	DP	27 m/s	+	60	+	70
4	DP	34 m/s	+	30	+	60
5	DP	27 m/s	+	40	+	70
6	DP	30 m/s	+	40	+	40
7	DP	23 m/s	+	70		
8	PBSt	16 m/s	+	60		
9	FDL		−		0	

Threshold for rubrospinal volley f om NR 30 μA.
Threshold for rubrospinal volley from MesADC 90 μA
Threshold fo PM-response from MesADC 30 μA.

effects evoked from the two central regions, while 3 cells were kept only for short perio time and not fully tested The threshold for evoking a rubrospinal volley from the elec just ventrally to the NR was about 30 μA From the electrode placed in MesADC a rubrospinal volley could be evoked at stimulating strengths above 90 μA. A PM-resp was, on the other hand, evoked already at 30 μA It is clear from the Table that all ef on the different cells were obtained at stimulating intensities which makes spread of cu from one region to the other unlikely This conclusion is supported by the fact that cell which was reciprocally influenced from the two regions at low stimulating intensities, controlled in the same way but stronger when stimulation was increased to 90 μA in and to 80 μA in MesADC The central effects on all cells included in the material prese have been studied under similar circumstances.

A cell influenced in the same reciprocal way as the FDL γ (cell 1) in Table I but belong to the plantaris muscle is illustrated in Fig. 2. This cell was slightly difficult to identify a responded antidromically from the nerve together with another cell (A). From the records in A and B it appears, however that when a spontaneous spike of the cell occurred clos enough to the antidromic stimulus (B) a collision of orthodromic and antidromic spikes to place in the axon and the antidromic response disappeared. The remaining smaller o belonging to the same nerve was not spontaneously active, nor was it influenced by the centr stimuli. The rubrospinal volley evoked from the NR-electrode is illustrated in C, and th characteristic PM-response from the MesADC-electrode in D (note lack of volley in Th 13 In Fig. 2 E is seen a strong inhibitory influence on the spontaneously active cell from NI at low stimulus intensity In F the cell is instead shown to be strongly activated from Me ADC. In this experiment the thresholds for detectable descending rubrospinal volley from the NR-electrode and for PM response from the MesADC-electrode were both aroun 40 μA. A weak rubrospinal volley for MesADC-stimulation was seen at 100 μA.

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. Effects from NR and MesADC on whole material of 63 cells.

...not influenced from MesADC (Static cells)

...m NR	GS	FDL	P	ABSm	PBSt	DP	Extensor	Flexor
...m	5	0	0	1	9	5	6	14
...n	3	1	1	0	0	0	7	0
.	10	1	1	1	9	5	13	14

...influenced from MesADC (Dynamic cells)

...m NR	GS	FDL	P	PBSt	DP	Extensor	Flexor
...m	1	0	0	5	4	1	9
...n	3	3	2	0	0	8	0
...ct	4	0	0	0	1	4	1
...r	8	3	2	5	5	13	10

...ls not tested from MesADC

...from NR	FDL	P	ABSm	Tib	PBSt	DP	Tens	Extensor	Flexor
...tion	1	1	1	1	3	4	1	4	8
...ion	0	0	0	0	0	1	0	0	1
...r	1	1	1	1	3	5	1	4	9

...ADC-system only causes selective dynamic effects, all cells activated from this region ...garded as dynamic fusimotor neurones (23 cells in B). Of the other cells 27 were found ...influenced from NR but not from MesADC and these are regarded as static neurones. The third group in C consists of 13 cells which were tested only from NR and thus could ...be characterized.

...study of Table II reveals the following interesting points.

...e majority of dynamic cells were influenced also from NR. This influence was almost ...tly reciprocal with excitation of flexor cells and inhibition of extensor cells (B).

...few dynamic cells were not reached by the rubrospinal tract (B).

...mong static cells to extensor muscles a mixture of excitation and inhibitory effects was ...nd while flexor cells were always excited (A).

...lso also the material of unclassified cells (unknown MesADC-influence) excitatory ...cts on the flexor neurones predominate. Of four extensor cells, on the other hand, all ...e excited leading to the suggestion that at least these cells were all static, as excitatory ...cts on extensor dynamic neurones were rare (C).

The ...naptic linkage between rubrospinal fibres and γ-motoneurones

...e technique of using long, continuous trains of stimuli in the central areas does not allow ...determination of the latency of the effects evoked. Attempts to use shorter trains of stimuli ...as often made. From NR it was frequently noted, that cells activated f om this region ...ere influenced by trains containing 4 or 5 pulses. This influence, however had the nature ...f irregular firing with greatly fluctuating latency from the effective shock and any exact ...ppreciation of the segmental latency was impossible.

In one experiment recording was made, not from cell bodies in the lumbar cord, but

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6	DP	30 m/s	+	40	+	40
7	DP	.3 m/s	+	70		
8	PBSt	16 m/s	+	60		
9	FDL		−		0	

Threshold for rubrospinal volley from NR 30 μA.
Threshold for rubrospinal volley from MesADC 90 μA.
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	5	1	1	0	0	0	7	0
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	3	3	2	0	0	8	0
	4	0	0	0	1	4	1
	8	3	2	5	5	13	10

...ot tested from MesADC

...n NR	FDL	P	AbSm	Tib	PBSt	DP	Ten	Extensor	Flexor
...	1	1	1	1	3	4	1	4	8
	0	0	0	0	0	1	0	0	1
	1	1	1	1	3	5	1	4	9

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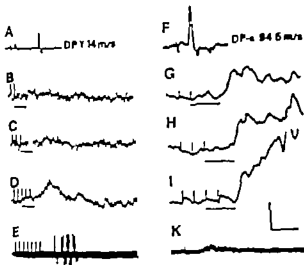
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6	DP	30 m/s	+	40	+	40
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9	FDL		−		0	

Threshold for rubrospinal olley from NR 30 μA.
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Intracellular recording from fusimotor (A–E) and intracellular recording from skeletomotor neurones both belonging to the deep peroneal nerve. In A and F the cells are antidromically identified.) and G–I postsynaptic potentials caused by varying number of stimuli to NR are shown. The horizontal line below each record denotes the distance between the actn stimulus and the resulting ... In E the fusimotor neurone is seen to start firing (superimposed records) to an increased number of stimuli. In K is shown the descending rubrospinal volley in L_4. Voltage calibration is 4 mV for A, 0.4 mV for B–D, 10 mV for F–I. Time: 10 ms for B–E, 4 ms for A and F K.

... in this experiment reached the L7 segment in 3.1 ms (Fig. 4 K shows the volley ...ded in L5 with a latency of 2.9 ms. About 0.2 ms should be added to account for the ...tional distance of 15 mm to the L7 segment). This lea es about 1.2 and 1.9 ms synaptic ... for the α- and the γ-cell respectively. Also this observation indicates that a disynaptic ...ing between the rubrospinal tract and α- as well as static γ-cells may occur (cf. Discus... During the course of the experiments a considerable number of intracellular recordings ... α-motoneurones were obtained. On each such occasion the effect on the cell from the ...stimulating electrode as well as from the MesADC was tested. It was consistently ...d that while the rubrospinal activation regularly caused postsynaptic potentials in these ... according to the pattern described by Hongo *et al.* (1969 a) α-motoneurones were never ...enced in any respect from the MesADC (cf. Discussion).

Conduction velocities of static and dynamic axons

...n the whole material of 63 cells the conduction velocities of their axons varied from ...o 55 m/s. For the 23 cells classified as dynamic the axon conduction elocity range was ...47 m/s with a mean of 31.1 m/s, and for 26 static cells the corresponding figures were ...35 m/s and 27.5 m/s. The tendency for the lowest conduction velocities to be found in ...ic neurones and the highest in dynamic ones is clear but not statistically significant. It is, ...ever partly in agreement with the observations made by Brown, Crowe and Matthews (...65) ...ho for the tibialis anterior muscle found that the slowest γ-fibres (in their study with ...duction velocities below 30 m/s) were virtually certain to be static. For the soleus muscle, ... the other hand, Crowe and Matthews (1964) found no such clear differences in conduction



Fig. 3. A, Identification · simus fusimotor neurone in nerve filament. In B three and four shocks activate α as well as γ neurone (upper traces). ing volley recorded in Th, in lower traces. For furthe see text. Time calibratio for A, 10 ms for B and 4 n

instead from axons in branches of the tenuissimus nerve. In this experiment a nerve fil was obtained containing a γ-fibre with a conduction velocity of 47 m/s (Fig. 3 A). Th was spontaneously active (*cf* arrows in A). The filament also contained some α-axons of these were activated together with the γ-fibre from the L7 ventral root (Fig. 3 A), a belonged to another ventral root The γ-fibre as well as the unidentified α-fibre were activated from NR by a short train of stimuli. From the analysis of a number of s imposed records of the type illustrated in Fig. 3 B and C it became clear that the numb pulses required in the NR to activate the γ-axon varied from record to record. Ca measurements of intervals between different stimulating pulses and the resulting bur spikes did, however reveal that the minimal latency from active pulse to fired γ-spike 7 8 ms. (In seven records each consisting of 10 or 20 superimposed sweeps this latency t burst was found from the second pulse in four cases and from the first, the third or fourth pulse in one case each) In Fig. 3 B and C the cell is in both cases fired by the sec rubral pulse. In this experiment activity in the fastest rubrospinal fibres reached the Th segment in 2.0 ms (*cf* arrows in Fig. 3 C, lower trace) and the L7-segment in 2.5 ms (a tional distance 60 mm). Thus 5.3 ms remains for segmental synaptic delay and central peripheral conduction. Conduction through the ventral root and the nerve could be cal lated on the basis of the known conduction velocity of the fibre and the measured dista and was 3.5 ms. This leaves a segmental delay of 1 8 ms for the activation of this γ-unit. T seems to imply a disynaptic linkage from the rubrospinal tract to this unit (*cf* Discussio

More conclusive evidence concerning the synaptic linkage between the rubrospinal tr and α as well as γ-motoneurones was obtained in recordings from cells belonging to t DP-nerve in another experiment (Fig. 4) In both cells, the α conducting at 94.5 m/s and t γ (classified as static and probably being recorded from in a juxtacellular position) at 14 m occasional EPSP:s could be evoked by two NR-pulses (B and G). Usually however thr pulses were required to regularly depolarize the cells (C and H). When four or five stimu were used these caused additional depolarization by their EPSP:s summating to the or caused by the third pulse (D and J) At still longer trains the γ-cell started to fire as illustrate by the superimposed record in E. The latency from the active stimulating pulse to the re sulting EPSP was for the α-cell 4.3 ms, for the γ-cell 5 0 ms. The descending rubrospin

e two functional classes, static and dynamic cells. The method adopted for this was upon previous findings that the MesADC-region, i.e. the area just dorsally to and ing into the dorsal part of the red nucleus, selectively influences the dynamic sensi- of muscle spindles (cf. Appelberg 1967, Appelberg and Jeneskog 1972 and also Appel- and Emonet-Dénand 1965 who showed that effects on extensor as well as flexor spindles obtained from the same area). The work of Appelberg and Jeneskog (1972) is of special ... in this connection. During their experiments various types of influence on single ... could be observed from different levels in a stimulating electrode track passing ... the mesencephalon at a rubral level. It became quite clear that the effects evoked ... the area just dorsally to the red nucleus were always purely dynamic (in contrast to ... evoked from sites within the red nucleus, which were often mixed static/dynamic and, ... light of the present experiments, probably at times evoked via the rubrospinal tract). ...skog (1974 a) later made a detailed comparison between the mesencephalic area ...ng dynamic spindle effects and the one evoking a D-zone response in the cerebellar ...edian lobule and found a near to perfect coincidence between the two. In the present ...ments the MesADC-electrode was placed with such a PM-response as a guide. At the time it was carefully controlled that no rubrospinal descending volley was evoked the chosen electrode site. With all this in mind we feel it fully justified to use such a ADC-stimulus as a methodological tool enabling the separation of γ-motoneurones their two functional classes: static and dynamic.

...pting the method of classification leads to an important conclusion, namely that ... dynamic γ-motoneurones are also controlled by the rubrospinal tract. From this ... that the rubrospinal system may be thought of as a motor system working with ... between α- and possibly both classes of γ-motoneurones. Nothing is known, how- ..., about the synaptic coupling between the tract and dynamic neurones. It may be of ...al relevance also to note that of 23 cells classified as dynamic 5 were not influenced ... the NR (Table II B). This may indicate that there exists a population of dynamic γ- ...oneurones not participating in α- γ-linked control but rather being private to the specia- ... dynamic control system. Whether other such subpopulations of dynamic γ-motoneu- ... exist possibly being private to other descending or reflex systems is at present not ...n. Appelberg and Jeneskog (1972) did, however, note that not all spindles seemed to ... their dynamic sensitivity controlled by the MesADC system.

... should finally be pointed out, that the observations of lack of any effect on α-moto- ...rones from the MesADC (while synaptic potentials were readily evoked from the NR) ... of considerable importance. They do strengthen the notion that the MesADC-system, ...ch according to Jeneskog (1974 a, b) may be a rubro-bulbo-spinal system with its origin ... the rostral parts of the NR, is a system exclusively controlling one type of motoneurones, ... dynamic fusimotor ones. Whether this system may also have other neuronal connections ... the spinal cord is not known. In fact a descending volley reminding of the one caused by ... dorsal reticulo-spinal system (Baldissera et al. 1972 a) was sometimes observed when ...mulating in the MesADC. The possible identity of the MesADC-system and the dorsal ...culo-spinal system remains an object for further investigation.

This work was supported by the Swedish Medical Research Council, Project No. 3473.

velocities between static and dynamic γ-axons. With such differences between indi muscles it is clear that the matter of conduction velocities should not be too much e sized in a material obtained from several muscles.

Discussion

The primary aim of the present investigation was to study the effects of the rubro tract on γ-motoneurones. According to Baldissera *et al* (1972 a and b) segmental rubro effects may be studied in isolation after a bilateral ablation of the sensory-motor cort the stimulating electrode is placed so as to activate interposito-rubral fibres just ventra the NR These precautions were taken in the present experiments and it seems safe to clude that the effects studied were truly rubrospinal. Within the whole material of 30 ex cells 11 were excited, 15 inhibited and 4 not influenced from the NR Of 33 flexor ce were excited, 1 inhibited and 1 not influenced. In a corresponding material of intracell recorded α-motoneurones (Hongo *et al* 1969 a, their Table I) 45 out of 57 flexor m neurones were purely excited none were purely inhibited while 12 received a mixtu excitation and inhibition Of 125 extensor motoneurones 50 were excited, 37 inhibited 38 influenced in a mixed way Although our material is smaller and obtained by extracel recording it is immediately evident that there is a close correspondence in the patter rubrospinal control of α- and γ-motoneurones.

Having in mind the concept of α- γ-linkage it is, however clearly not enough to dem strate a similarity in effect on the two groups of motoneurones from a certain source. also necessary to demonstrate an equality in minimal synaptic linkage. That the rubros tract fibres may contact α-motoneurones via a single interneurone seems well establi (Hongo *et al* 1969 a and verified in the present investigation).

In the present work on γ-motoneurones, on the other hand, careful determination of t for central delay was arrived at only twice. For the juxtacellulary recorded DP-cell this d was 1 9 ms, a figure possibly allowing a passage through three synapses. It is possi however to suggest a disynaptic linkage and ascribe the seemingly long delay to the po bility that the descending fibre activating the cell had a somewhat slower conduction velo than the fastest ones causing the very beginning of the descending volley

For the γ-fibre recorded in the tenuissimus muscle nerve the central delay could be c culated with satisfactory accuracy to be 1 8 ms. In this case, however the time arrived includes spike initiation and the disynaptic nature of the coupling can hardly be doubt The fact that the cell in one recording actually responded to the first shock in NR may attributed to the fact that the cell was spontaneously active and therefore sometimes e tremely easily triggered

In conclusion it is, therefore, provisionally suggested that the synaptic coupling betwe the rubrospinal tract and α- as well as static γ-motoneurones is minimally disynaptic. The by the rubrospinal tract is added to the list of descending systems theoretically capable providing α γ-linked movement. The vestibulo-spinal tract as well as the MLF-system we previously shown to be organized in this manner (*cf* Grillner 1969).

The second aim of the experiments was to attempt to divide the material of γ-motoneuron

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From the Department of Physiology University of Goteborg, Sweden

A Method for the Continuous Study of Net Water Transport in the Feline Small Bowel

By

MATS JODAL, DAN-AXEL HALLBÄCK, JOAR SVANVIK and OVE LUNDGREN

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Abstract

M., D.-A. HALLBÄCK, J. SVANVIK and O. LUNDGREN. *A method for the continuous study of net water transport in the feline small bowel.* Acta physiol. scand. 1975. 95. 441–447.

perfusion technique has been developed for the study of net water transport across the intestinal ... in vivo. The lumen of an isolated intestinal segment is steadily perfused with a solution of known ... in a closed perfusion system with a reservoir large enough to prevent recirculation. The ... segment may be enclosed in a plethysmograph. Changes in the perfused volume is recorded by ... transducer coupled to the recirculating system via a T-tube. If no motility occurs, the changes of the ... volume reflects net water transport across the intestinal epithelium. A quantitative comparison ... technique with the conventional polyethylene glycol method revealed no significant difference. The ... graphic method also makes it possible to quantify the net water absorption via lymph and blood.

... number of methods have been developed for the study of net water transport across ... intestinal epithelium in animals and man (for review see Parsons 1968). One of the most ... in vivo techniques involves the use of a solution containing an inert, nonabsorbable ..., often polyethylene glycol. The solution is infused in one part of the intestine and ... ted downstream. An increase (decrease) of the concentration of the inert tracer during ... passage through the intestine reflects a net water absorption (secretion), which can be ... fied. Adding radioactively labelled water to the perfusion medium also enables the ... of unidirectional fluxes of water across the gut epithelium. However with this method ... impossible to follow transient changes of net water absorption occurring within minutes, ... it is necessary to perfuse the intestinal segment during a fairly long time period to ... a steady state situation, as regards the tracer. To circumvene these difficulties in the ... thetized animal, a perfusion method has been developed which allows a continuous ... mation of net water transport across the epithelium of the feline small bowel.

Methods

... *operative procedures and determination of blood flow.* Experiments were performed on 25 cats of both ... weighing 2–4 kg, anaesthetized intravenously with ... mg/kg b.w.) after induction with

References

APPELBERG B., The effect of electrical stimulation in nucleus ruber on the response to stretch in p and secondary muscle spindle afferents. *Acta physiol. scand.* 196_. 56. 140–151

APPELBERG, B., A rubro-oli ary pathway II Simultaneous action on dynamic fusimotor neurones activity of the posterior lobe of the cerebellar cortex. *Exp Brain Res.* 1967 3 382 390.

APPELBERG, B and F EMONET DENAND Central control of static and dynamic sensitivities of spindle primary endings. *Acta physiol scand.* 1965 63 487–494

APPELBERG, B. and I Z. KOSARY Excitation of flexor fusimotor neurones by electrical stimulation red nucleus. *A ta physiol. scand.* 1963 59 445–453.

APPELBERG, B. and T JENESKOG, Mesencephalic fusimotor control. *Exp. Brain Res.* 197_ 15 97-1

BALDISSERA, F A. LUNDBERG and M UDO, Activity evoked from the mesencephalic tegmentum scending pathways other than the rubrospinal tract *Exp. Brain Res.* 1972 a. 15 133–150.

BALDISSERA, F A. LUNDBERG and M UDO, Stimulation of pre- and postsynaptic elements in nucleus. *Exp Brain Res.* 1972 b. 15 151 167

BROWN, M C A CROWE and P B. C. MATTHEWS, Observations on the fusimotor fibres of the posterior muscle of the cat *J Physiol.* (Lond.) 1965 177 140–159

CROWE, A. and P B. C. MATTHEWS, Further studies of static and dynamic fusimotor fibres. *J* (Lond) 1964. 174 132 151

GRILLNER, S S praspinal and segmental control of static and dynamic γ-motoneurones in the c *physiol scand* 1969 Suppl. 327

HONGO T ELŻBIETA JANKOWSKA and A LUNDBERG, The rubrospinal tract. I Effects on alpha neurones innervating hindlimb muscles of the cat. *Exp Brain R s.* 1969 a. 7 344–364.

HONGO, T ELŻBIETA JANKOWSKA and A LUNDBERG, The rubrospinal tract. II. Facilitation of intern transmission in reflex paths to motoneurones. *Exp Brain Res.* 1969 b. 7 365–391

HONGO, T ELŻBIETA JANKOWSKA and A. LUNDBERG, The rubrospinal tract. III. Effects on pri ferent terminals. *Exp. Brain Res* 1972 a. 15 39–53

HONGO, T ELŻBIETA JANKOWSKA and A. LUNDBERG, The rubrospinal tract IV Effects on interne *Exp. Br in R s.* 1972 b. 15 54–78.

JENESKOG T Parallel acti ation of dynamic fusimotor neurones and a climbing fibre system from brain stem. I Effects from the rubral region. *Acta physiol. scand.* 1974 a. 91 223– 42.

JENESKOG, T Parallel activation of dynamic fusimotor neurones and a climbing fibre system from brain stem. II Effects from the inferior olivary region. *Acta phy iol. scand.* 1974 b. 92. 66–83.

SASAKI, K. A. NAMIKAWA and S. HASHIRAMOTO, The effect of midbrain st mulation upon alpha neurones in lumbar spinal cord of the cat *Jap J Phy iol.* 1960. 10. 303–316.

and 2), recording on Grass polygraph. Venous outflow pressure could be set at any desired ... of the end of the tube draining the drop recorder. It was usually kept around 10 mm ... blood was returned to the animal via a funnel and catheter in the right jugular vein. In ... the main lymph vessel draining the intestinal segment and its lymph nodes was cannulated ... In the rest of the experiments the lymph vessels were left untouched or cut open. ... carefully avoided during the operative procedures and, if occurring, substitution by ... body-warm Dextran-Tyrode solution was performed. Body temperature was maintained ... °C by means of thermostatically regulated heating pad.

... *and plethysmographic techniques.* In all experiments the jejunal lumen was perfused at ... with solution of known composition (see below). To make this possible the jejunal segment ... to recirculating system with a large perspex reservoir containing about 900 ml of fluid ... The fluid was perfused at constant rate (usually around 1 ml/min) through the intestinal ... of roller pump (Ismatec SA, Zürich, Switzerland, Model mp-ge), the temperature of ... fluid entering the segment being continuously recorded by thermocouple thermometer ... Copenhagen) and kept at 38°C by heating pad. To promote mixing in the reservoir and ... recirculation, the perspex reservoir was divided into number of interconnected smaller com... The recirculation system was connected to volume transducer via T-tube, continuously ... in the recirculation volume (Fig. 1 and 2). The pressure at the outflow tubing could be ... of the height of the volume transducer procedure which influenced net water ab... (Dennhardt and Haberich 1971). The outflow pressure was usually kept at the lower end of ... range, i.e. 1–2 cm H_2O.

... experiments the intestinal segment was also enclosed in triangular perspex plethysmograph ... and sensitive recording of the volume of the jejunal segment and its lymph nodes ... was made possible by placing the wide-bore cannula in the mesenteric vein at the proximal ... of the plethysmograph, thereby avoiding venous collapse by undue lateral pressure (Folkow ... This opening in the plethysmograph was sealed by silicone grease (Dow Corning). The plethys... filled with modified Krebs-Henseleit solution (see below) and connected to volume trans... on Grass polygraph. The temperature of the plethysmograph was maintained at 38°C, ... controlled by thermocouple thermometer (Electrolab, Copenhagen). The polyethylene ... the jejunal segment to the perfusion system fitted closely through openings in the ... wall, making it possible to perfuse the lumen of the intestinal segment in the ... plethysmograph.

... were also performed in which the intestinal segment was not enclosed in the plethysmograph. ... set up was then otherwise identical to that described above for the plethysmographic ... (Fig. 2).

Polyethylene glycol method. Net water transport was in some experiments determined by means ... polyethylene glycol (PEG molecular weight around 4 000; purchased from New England Nuclear ... West Germany or from Radiochemical Centre, Amersham, England). To 100 ml of the ... solution 0.5 µCi of PEG was added. When the perfusion through the intestinal segment had been ... during steady state" for such long time that the perfused volume amounted to at least ... volume assumed to be contained in the gut lumen, 200 µl samples were taken from the inflow ... outflow of the intestinal segment. After adding 8 ml of Instagel® (Packard), radioactivity was ... Packard Tricarb Liquid Scintillation Spectrometer, model 3320 (counting efficiency around ... quenching being controlled by the external standard and/or the channel ratio method). The ... rate of the recirculation system was checked before and after each experimental run. Net water ... was calculated from the change of concentration of the tracer during its passage through the ...

... *acid-base balance of the experimental animal.* In order to maintain fairly constant arterial pH ... throughout the experiments, bicarbonate solution (10 mmol $NaHCO_3$ per 100 ml 10 % glucose ... was infused at rate of 0.1 ml/min during the experiments. This procedure was earlier de... to maintain arterial pH and P_{CO_2} at normal and constant level, despite the surgical trauma ... and Lundgren 1972).

... In most experiments the perfusion solution contained (mmoles/l, pro analysi): NaCl 122, ... $NaHCO_3$ 25, KH_2PO_4 1.2, $MgCl_2\cdot6H_2O$ 1.2, Glucose 30, $CaCl_2$ 2.5. Osmolality of this solution ... 316 mOsm/kg H_2O as determined by an Advanced Instruments osmometer and plasma osmolality ... of the same order of magnitude (i.e. 316±10 mOsm/kg). If plasma osmolality exceeded 330, ... of the perfusion solution was adjusted to plasma osmolality by the addition of appropriate

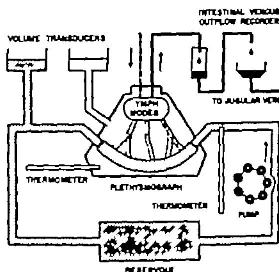


Fig. 1 Schematic illustration of the plethysmographic technique used for studying net water across the intestinal epithelium as well as net water absorption *via* lymph and blood.

ether The cats had been depri ed of food for 24 h w th free access to water and exhibited no ob of intestinal disease.

The operative procedures were largely similar to those used in previous studies on the intestinal in this laboratory (*cf* Folkow *et al* 1963, Kampp *et al.* 1968). The abdomen was opened in t and the greater omentum and the spleen were ext rpated. A segment of the jejunum, about 10 the Treitz's ligament and weighing 10–20 g, was isolated and the remainder of the intestinal tract The lumen of the jejunal segment was flushed with bodywarm saline until the leaving fluid was c care was taken to keep the intestinal segment moistened throughout the operati e procedures by gauze soaked in saline. The influence of the sympathico-adrenal system was eliminated by bilate ing of the splanchnic nerves, denervating also the adrenal glands. The vagal influence was elim i.a. administered atropine, 1 mg/kg b.w

After heparinization the right femoral artery was connected to a Statham pressure transducer to record mean arterial pressure. The superio mesenteric vein draining all blood from the jejun and its lymph nodes, was cannulated and connected to an optical drop recorder unit operating

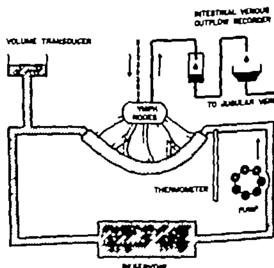


Fig. 2. Schematic illustration of the perfusion system used for recording net water transport in

(Fig. 1 and 2), recording on [illegible] Grass polygraph. Venous outflow pressure could be set at any desired [illegible] by adjustment of the end of the tube draining the drop recorder. It was usually kept around 10 mm [illegible]. The venous blood was returned to the animal [illegible] funnel and [illegible] catheter in the right jugular vein. In [illegible] experiments the main lymph vessel draining the intestinal segment and its lymph nodes was cannulated [illegible] possible. In the rest of the experiments the lymph vessels were left untouched or cut open. [illegible] was carefully avoided during the operative procedures and, if occurring, substitution by [illegible] of body-warm Dextran-Tyrode solution was performed. Body temperature was maintained [illegible] at 38 C by means of [illegible] thermostatically regulated heating pad.

Perfusion and plethysmographic techniques. In all experiments the jejunal lumen [illegible] perfused at [illegible] with [illegible] solution of known composition (see below). T[illegible] make this possible the jejunal segment [illegible] connected to [illegible] recirculating system with [illegible] large perspex reservoir containing about 900 ml of fluid [illegible] 1 and 2). The fluid [illegible] perfused at [illegible] constant rate (usually around 1 ml/min) through the intestinal [illegible] by means of [illegible] roller pump (Ismatec Sa, Zürich, Switzerland, Model mp-ge), the temperature of [illegible] fluid entering the segment being continuously recorded by [illegible] thermocouple thermometer [illegible], Copenhagen) and kept at 38°C by [illegible] heating pad. T[illegible] promote mixing in the reservoir and, [illegible] recirculation, the perspex reservoir was divided into [illegible] number of interconnected smaller com[illegible]. The recirculation system was connected to [illegible] volume transducer via [illegible] T-tube, continuously [illegible] changes in the recirculation volume (Fig. 1 and 2). The pressure at the outflow tubing could be [illegible] by adjustment of the height of the volume transducer, [illegible] procedure [illegible] which influenced net water ab[illegible] rate (Dresbach and Haberich 1971). The outflow pressure was usually kept at the lower end of [illegible] physiological range, i.e. 1–2 cm H_2O.

[illegible] experiments the intestinal segment was also enclosed in [illegible] triangular perspex plethysmograph [illegible] continuous and sensitive recording of the volume of the jejunal segment and its lymph nodes [illegible]. This was made possible by placing the [illegible] wide-bore cannula in the mesenteric vein at the proximal [illegible] of the plethysmograph, thereby [illegible] avoiding venous collapse by undue lateral pressure (Folkow [illegible] 1963). The opening in the plethysmograph was sealed by silicone grease (Dow Corning). The plethys[illegible] was filled with [illegible] modified Krebs-Henseleit solution (see below) and connected to a volume trans[illegible] recording on [illegible] Grass polygraph. The temperature of the plethysmograph [illegible] was maintained at 38°C, [illegible] continuously controlled by [illegible] thermocouple thermometer (Electrolab, Copenhagen). The polyethylene [illegible] connecting the jejunal segment to the perfusion system fitted closely through openings in the [illegible] plethysmographic wall, making it possible to perfuse the lumen of the intestinal segment in situ inside [illegible] plethysmograph.

[illegible] experiments were also performed in [illegible] which the intestinal segment was not enclosed in the plethysmograph. [illegible] experimental set up was then otherwise identical to that described [illegible] above for the plethysmographic [illegible] (Fig. 2).

[illegible] ^{14}C-Polyethylene glycol method. Net [illegible] water transport was in some experiments determined by means [illegible] ^{14}C-polyethylene glycol (PEG- molecular weight around 4 000; purchased from New England Nuclear [illegible], West Germany or from Radiochemical Centre, Amersham, England). T[illegible] 100 ml of the [illegible] solution 0.5 μCi of PEG was added. When the perfusion through the intestinal segment had been [illegible] during [illegible] "steady state" for such [illegible] long time that the perfused volume amounted to at least [illegible] the volume assumed to be contained in the gut lumen, 200 μl samples [illegible] were taken from the inflow [illegible] the outflow of the intestinal segment. After adding 8 ml of Instagel® (Packard), radioactivity was [illegible] [illegible] a Packard Tricarb Liquid Scintillation Spectrometer model 3320 (counting efficiency around [illegible] per cent, quenching being controlled by the external standard and/or the channel ratio method). The [illegible] rate of the recirculation system was checked before and after each experimental run. Net water [illegible] was calculated from the change of concentration of [illegible] the tracer during its passage through the [illegible].

[illegible] The acid-base balance of the experimental animal. In order to maintain [illegible] fairly constant arterial pH [illegible] P_{CO_2} throughout the experiments, [illegible] bicarbonate solution (10 mmol $NaHCO_3$ per 100 ml 10 [illegible] glucose [illegible] was infused [illegible] at [illegible] rate of 0.1 ml/min during the experiments. This procedure was earlier de[illegible] to maintain arterial pH and P_{CO_2} at normal and constant level, despite the surgical trauma [illegible] and Lundgren 1972).

[illegible] Solutions. In most experiments the perfusion solution contained (mmoles/l, pro analysi): NaCl 121, [illegible] $NaHCO_3$ 25 KH_2P[illegible] 1.2, $MgCl_2 \cdot 6H_2O$ 1.2, Glucose 30 $CaCl_2$ 2.5. Osmolality of this solution [illegible] 314 mOsm/kg H_2O as determined by an Advanced Instruments' osmometer and plasma osmolality [illegible] of the same order of magnitude (i[illegible] 316 ± 10 mOsm/kg). If plasma osmolality exceeded 330, [illegible] osmolality of the perfusion solution was adjusted t[illegible] plasma osmolality by the addition of appropriate

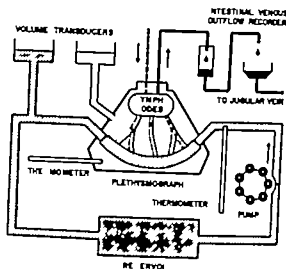


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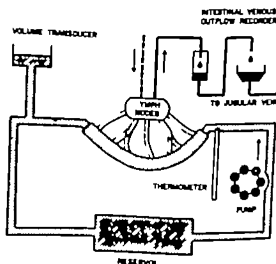


Fig. 2. Schematic illustration of the perfusion system used for recording net water transport in the intestine of the cat.

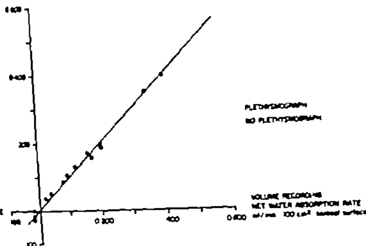


Fig. 4 Correlation between net water transport rate determined by means of polyethylene glycol (PEG) and by the perfusion methods depicted in Fig. 1 and 2. Line denotes eq. y = 0.955x − 0.007 determined by the method least squares. r = 0.94 (n = 33).

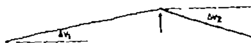


Fig. 5 The mode of analysis of the plethysmographic recording, schematically illustrated. At arrow the plasma transducer of the perfusion system (see Fig. 1) was clamped. For details, see text!

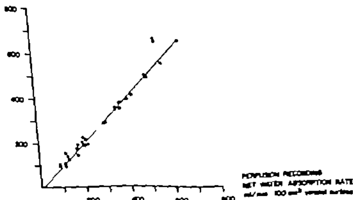


Fig. 6 The correlation between net water absorption rate determined from the decline of perfusion volume and calculated from the change in plethysmographic volume (see text). Line denotes eq. y = 1.069x − 0.01 calculated by the method of least squares. r = 0.98 (n = 41).

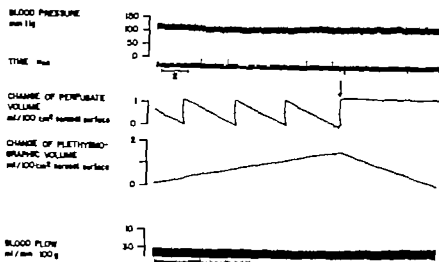


Fig. 3 Original tracing from experiment using the plethysmographic method of Fig. 1 "Start" de addition of 0.5 ml to the volume transducer of the perfusion system (left transducer of Fig. 1). At a volume transducer of the perfusion system was excluded from the recirculating system by means of Péan's forceps.

amounts of mannitol. The solution in the plethysmograph was identical to the abovementioned on that no $CaCl_2$ was added. In some experiments 5–10 μg of isoprenaline was added to the plethysmo solution (200 ml) to minimize motility

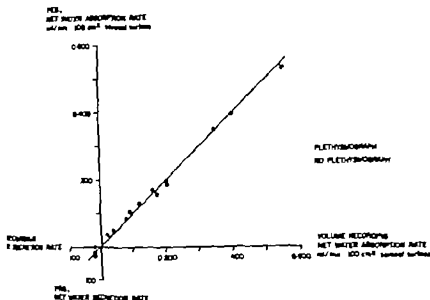
Results

The rate of net water absorption (or secretion) was in all experiments estimated fro continuous decrease of perfusate volume, recorded by the volume transducer as shown second upper panel of Fig. 3 In 13 expts. the rate of net water transport recorded b technique was compared with that estimated using ¹⁴C PEG The results of these experi demonstrated a close agreement between the two methods, as illustrated in Fig. 4.

In the plethysmographic experiments, an increase of the volume contained withi plethysmograph was regularly noted when the intestine was in the absorptive state. Wh absorption occurred, no increase of plethysmographic volume was recorded. How excluding the left volume transducer of Fig. 1 from the recirculating system by a p Péan's forceps reversed the increase of plethysmographic volume to a decrease as dep on Fig. 3 (arrow denotes clamping)

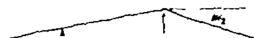
It was felt that the increase of plethysmographic volume illustrated in the left pa Fig. 3 was related to the absorption of water *via* the lymphatic vessels and that the decrea the right part of Fig. 3 reflected net blood absorption. For this reason, the plethysmogra recordings were analyzed as shown in Fig. 5 The rate of volume increase (ΔV_1) reco prior to clamping was added to the rate of volume decrease (ΔV_2) seen after clamping. sum of these two volume changes was compared to the simultaneously recorded net w absorption rate, calculated from the change of perfusate volume. Such a comparison, formed in 18 experiments at different levels of absorption rate, revealed a close correla between the two methods as illustrated in Fig. 6.

Furthermore, in some plethysmographic experiments it was possible to cannulate on

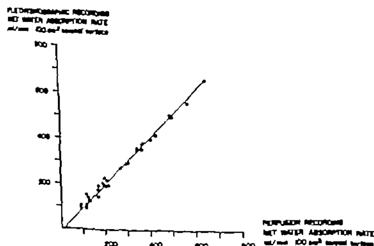


Correlation between net water transport rate determined by means of polyethylene glycol (PEG) the perfusion methods depicted in F g. 1 and 2. Line denotes eq. y 0.933 0 007 determined by and least squares. $_{xy}$ 0.94 (n 33)

CHANGE OF PLETHYSMOGRAPHIC VOLUME



The mode of analysis of the plethysmographic recording, schematically illustrated. At arrow the transducer of the perfusion system (see Fig. 1) was clamped. For details, see text.



6. The correlation between net water absorption rate determined from the decline of perfusion volume calculated from the change in plethysmographic volume (see text). Line denotes eq. y = 1.069x − 0 012 ained by the method of least squares. $_{xy}$ = 0 98 (n 41).

TABLE I Comparison between lymph flow expressed in ml/min, estimated from change of plethygraphic volume and from volume of lymph collected *via* a lymphatic cannula.

Plethysmographic recording	Collected lymph
0.0248	0.0239
0.0096	0.0113
0.0016	0.0015
0.0017	0.0014

the lymphatic vessels draining the intestine and its lymph nodes. Lymph flow could then determined by collecting lymph during a 15–20 min period This flow value was compa with that determined from the increase of plethysmographic volumes seen after occlu the lymph cannula The data obtained in 4 expts. are shown in Table I. No signific difference between the two set of values was found.

Quantitatively net water absorption rate at "resting" intestinal blood flow amounted 0.25–0 50 ml/min 100 cm serosal surface, when the intestine was isovolumetric and int luminal pressure at the outflow end of the intestine was 1–2 cm H_2O 20–40% of this volu was absorbed *via* the lymphatic vessels. A detailed quantitative account of these experime will be given in a subsequent report

Discussion

This paper presents a simple method making it possible to study continuously the net r of water transport across the intestinal epithelium of an anaesthetized animal. A compari between this method and the conventional PEG-technique revealed a close correlat (Fig. 4). The major technical difficulty of the present technique is the presence of motil which was easily detected by inspection and/or by abrupt volume changes of the recor perfusate volume. To avoid motility various precautions were taken. First, the segm used in the experiments was a jejunal one situated about 10 cm below Treitz's ligament avoid the pacemaker regions in the most proximal part of the gut. Second, intraluma pressure was kept at the lower end of the physiological range, *i.e* at a pressure of 1 2 H O at the outflow end. Third, the plethysmographic solution was devoid of Ca, the pr ence of which is necessary for the initiation of smooth muscle contractions. Fourth, in so plethysmographic experiments, a small amount of isoprenaline, a potent smooth mus relaxing agent was added. Fifth great care was taken to keep the segment moistened and prevent cooling of the segment With these precautions taken, the intestine exhibited motility in most experiments.

It was regularly noted that the plethysmographic volume recording increased when water absorption occurred. In situations when one may assume that the intestinal tissue "isovolumetric" this increase in all probability reflects the absorption of water *via* lympha vessels. This fluid absorbed was then "trapped in the plethysmograph since it could r overcome the high sealing pressure at the plethysmographic exit Since the lymphatic vess of the cat mesentery are almost devoid of smooth muscle cells (Yoffey and Courtice 197 it seems reasonable to assume that the lymph can easily be filtered through the vascular w into the tissue. This interpretation is supported by four observations. 1 When no absorpti

...rated no increase of the plethysmographic volume was noted. 2. When excluding the left ...ansducer of Fig. 1 from the closed luminal perfusion system a decrease of plethys... volume was recorded (Fig. 3), probably reflecting net water absorption of fluid ...ood. The amount of fluid absorbed from the perfusion system was then derived ...ole compliant part, *i.e.* the intestine, and fluid absorbed *via the lymph remained* ...thysmograph. 3. Adding together the rate of *increase* in plethysmographic volume during water absorption, and the rate of *decrease* recorded after clamping the ...ansducer of the perfusion system (Fig. 5), one always arrived at a value which was ...the rate of total net water absorption (Fig. 6). 4. Measurements of lymph flow from ...d intestinal lymph vessels compared well with the volume increase recorded by the ...ograph after clamping the lymph cannula (Table 1).

...ld be pointed out that the present technique enables not only the study of net ...sport but, by addition of proper tracers, bidirectional fluxes of water or any other ... can easily be followed. In such experiments samples are taken of the fluid entering ...ing the intestinal segment.

...ch ...as sponsored by grants from the Swedish Medical Research Council (14X-...55), from ...d Gösta Janssons stiftelse, from Clara and Lilly Dahlströms fond, from Wilhelm and Martina ... Vetenskapsfond, from *Magnus Bergvalls stiftelse* and from the Medical Faculty, University ...rg.

References

...z, R. and F. J. HABERICH, Resorptions- und Sekretionsstudien am Darm. V. Einfluss des intra-...en Drucks auf die Wasserresorption am Duodenum wacher Ratten. *Z. ges. exp. Med.* 1971. 156. ...

...L, O. LUNDGREN and I. WALLENTIN, Studies on the relationship between flow resistance, capil-...ation coefficient and regional blood volume in the intestine of the cat. *Acta physiol. scand.* 1963. ...–283.

..., U. and O. LUNDGREN, Reactions within consecutive vascular sections of the small intestine ...at during prolonged hypotension. *Acta physiol. scand.* 1972. 84. 151–163.

...M., O. LUNDGREN and J. ENGSTRAND, On the components of the Kr^{85} wash-out curves from the ...intestine of the cat. *Acta physiol. scand.* 1968. 72. 257–286.

... D. B., Methods for investigation of intestinal absorption. *In: Handbook of Physiology* 1968, ...6 (Alimentary Canal), vol. III. 1177–1216.

... J. M. and F. C. COURTICE, *Lymphatics, lymph and lymphomyeloid complex.* Academic Press, London ...w York, 1970.

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Acylation of Carnitine and Glycerophosphate in Suspensions of Liver Mitochondria at Varying Levels of Palmitate and Coenzym

By

B. BORREBAEK

Received 23 May 1975

Abstract

BORREBAEK, B *Acylation of carnitine and glycerophosphate in suspensions of rat liver chondria at varying levels of palmitate and coenzyme A* Acta physiol. scand. 95 448–456.

Rates of acylation of carnitine and glycerophosphate in suspensions of isolated rat liver mitoch were measured at varying levels of palmitate and coenzyme A in the presence of ATP Addition of gl phosphate caused considerable reduction in carnitine acylation at low but not at high palmitate l Glycerophosphate acylation was less reduced by added carnitine. These results can be explained lower K_m(acyl-CoA) in glycerophosphate acylation than in carnitine acylation. High levels of free coen A caused inhibition of carnitine acylation, while the inhibitory effect of glycerophosphate acylation small. Competition between palmitate and acyl-CoA for binding sites on protein (e g albumin add the mitochondrial suspensions) was indicated by stimulation of carnitine acylation by palmitate palmityl-CoA added as substrate n the absence of ATP Refeeding a carbohydrate-rich diet to previ fasted rats resulted in increased glycerophosphate acylation and decreased carnitine acylation in bo liver mitochondria.

The rates of oxidation and esterification of fatty acids in the liver are dependent on nutritional state of the animal. A rapid inhibition by glucose of fatty acid oxidation *vice versa* have been demonstrated (Lossow and Chaikoff 1955 Randle *et al* 1963, Sh 1964), and it has been shown that the glucose effect is correlated with the tissue level long-chain acylcarnitines (Bohmer 1967 Pearson and Tubbs 1967) This level is at least part the result of an increased concentration of free fatty acids in blood plasma, leading increased uptake of fatty acids by the tissues. However perfused livers from fasted r oxidize more fatty acids to ketone bodies and carbon dioxide and esterify less to triglyceri than perfused livers from fed animals under identical conditions (Mayes 1970 McGar *et al.* 1973). It has been shown that the glycerophosphate acylating enzyme (acyl-Co sn-glycerol-3-phosphate *O*-acyltransferase, EC 2. 3 1 15) is reduced and that carniti palmityl transferase (acyl-CoA L-carnitine *O*-acyltransferase EC 2. 3 1 21) is increase in the liver when previously well fed rats are fasted (Norum 1965 Vavrecka *et al.* 196 Aas and Daae 1971). However it has not been established that these variations in enzym

[illegible] The acylation of carnitine and glycerophosphate by mitochondria from livers of fasted and fasted/refed rats. Rats were refed the carbohydrate diet. Carnitine acylation was performed in the presence of unlabelled glycerophosphate, and glycerophosphate acylation in the presence of unlabelled carnitine. The data, presented together with the S.E. values, represent means of 6–12 observations. The amount of added CoA was 30 nmol. No palmityl-CoA was added.

[illegible]	Mitochondrial protein of the whole liver (g)	Palmitate added (μM)	Carnitine acylation (nmol/min, mg protein)	Glycerophosphate acylation
[illegible] 48 h	1.07±0.09	[illegible]	10.45±1.21	3.19±0.3[illegible]
		220	2.83±0.09	2.75±0.14
[illegible] 48 h, [illegible] 4 h	1.12±0.11	[illegible]	9.20±0.95	3.47±0.27
[illegible] 48 h, [illegible] 10 h	1.03±0.12	[illegible]	6.40±0.40	6.82±0.51
[illegible] 48 h, [illegible] 4 h	1.10±0.10	[illegible]	4.72±0.32	3.55±0.66
		220	1.82±0.24	3.48±0.16

[illegible] can explain the dietary variations in fatty acid oxidation and esterification. The [illegible] of acylcarnitines in the liver depends on the rates of fatty acid activation (catalyzed [illegible] long-chain fatty acid. CoA ligase (AMP-forming) EC 6. 2. 1. 3), glycerophosphate [illegible]tion and carnitine acylation. Isolated mitochondria contain the three enzymes catalyzing [illegible] processes (Farstad *et al.* 1967 Norum and Bremer 1967 Zborowsky and Wojtczak [illegible] Daae and Bremer 1970), and represent therefore a suitable and well defined model [illegible] for a kinetic study of this branching point in fatty acid metabolism.

[illegible] the present work, the kinetics of *carnitine acylation and glycerophosphate acylation* [illegible] suspensions of isolated mitochondria were studied with palmitate, CoA, ATP carnitine, [illegible] glycerophosphate as substrates. *It is shown that* carnitine acylation is inhibited by free [illegible] and stimulated by palmitate. It is further shown that increased glycerophosphate [illegible]tion and decreased carnitine acylation in the isolated mitochondria were the results [illegible] previously fasted rats were refed a carbohydrate diet.

Methods

[illegible] Male albino rats of the Wistar strain, weighing 100–200 g were used. The animals were supplied [illegible] Møllegård A [illegible], Havrup, Denmark. They were maintained on stock diet (containing 24 [illegible] protein, 23 crude carbohydrate, 2.6% fat, 5.6 fiber and 5 ash) obtained from Petter Larsen [illegible] Oslo, Norway, and free access to water. *Fasted* rats were deprived of food for 48 h. The fasted/[illegible] rats were fasted for 48 h and then given free access to 20 glucose in the drinking water and [illegible] of glucose and crumbed bread for the times indicated. The experiments were performed with [illegible] mitochondria from rats fed the stock diet, except those presented in Table I.

[illegible] The following compounds were obtained from *Sigma Chemical Company St. Louis, Mo.*, [illegible] ATP (disodium, cat. A 3127), DL-glycerophosphate (cat. G-6126), palmitic acid (cat. P-0500), palmityl-[illegible] (cat. P-1250), Coenzyme A (Lithium salt cat. C-9004), β-NAD (cat. N 7004), acetyl phosphate (cat. [illegible]), L-malic acid (cat. A-6003), phosphate acetyltransferase (Acetyl-CoA: ortophosphate acyl-[illegible] EC 2.3.1.8; 1320 Units/mg, 5 mg/ml, cat. P-4383), malate dehydrogenase (L Malate- NAD [illegible] EC 1.1.1.37 1300 Units/mg, 10 mg/ml, cat. 410-13), and citrate synthase (Citrate oxalo-

[illegible]

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From the Institute of Medical Biochemistry University of Oslo, Norway

Acylation of Carnitine and Glycerophosphate in Suspensions of R Liver Mitochondria at Varying Levels of Palmitate and Coenzym

By

B. Borrebæk

Received 23 May 1975

Abstract

Borrebæk, B *Acylation of carnitine and glycerophosphate in suspensions of rat liver chondria at varying levels of palmitate and coenzyme A* Acta physiol. scand. 95 448–456.

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The rates of oxidation and esterification of fatty acids in the liver are dependent nutritional state of the animal A rapid inhibition by glucose of fatty acid oxidati *vice versa* have been demonstrated (Lossow and Chalkoff 1955 Randle *et al* 1963, 1964), and it has been shown that the glucose effect is correlated with the tissue l long-chain acylcarnitines (Bohmer 1967 Pearson and Tubbs 1967). This level is at l part the result of an increased concentration of free fatty acids in blood plasma, lea increased uptake of fatty acids by the tissues. However perfused livers from fast oxidize more fatty acids to ketone bodies and carbon dioxide and esterify less to trigl than perfused livers from fed animals under identical conditions (Mayes 1970, M *et al* 1973). It has been shown that the glycerophosphate acylating enzyme (ac *sn*-glycerol-3-phosphate *O*-acyltransferase EC 2. 3 1 15) is reduced and that ca palmityl transferase (acyl-CoA L-carnitine *O*-acyltransferase EC 2. 3 1 21) is inc in the liver when previously well fed rats are fasted (Norum 1965 Vavrecka *et al* Aas and Daae 1971) However it has not been established that these variations in c

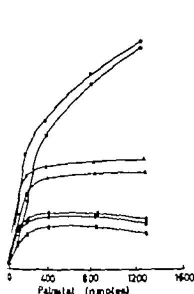


Fig. 1

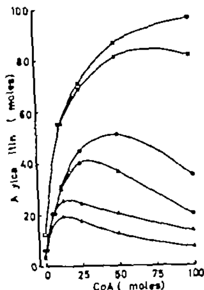


Fig. 2

Carnitine acylation, glycerophosphate acylation, and the level of acyl-CoA at different amounts of palmitate. Products are 1: acylcarnitine formed from labelled carnitine the absence ( ) and presence of unlabelled glycerophosphate, 2: acylated glycerophosphate formed from labelled glycerophosphate in the absence (△) and presence (▲) of unlabelled carnitine, and 3: acyl-CoA in the presence of carnitine ( ), glycerophosphate ( ), or both (●). The amount of added CoA was 50 nmoles. No palmityl-CoA was added.

Carnitine acylation at different amounts of added CoA. Open symbols indicate the absence and filled the presence of unlabelled glycerophosphate. The amounts of added palmitate were 880 nmoles, 220 nmol (○, ●), and 110 nmol (▲). No palmityl-CoA was added.

Results

Increases in carnitine acylation and glycerophosphate acylation with increasing palmitate levels are shown in Fig. 1. Carnitine acylation was depressed approximately 50 % when unlabelled glycerophosphate was added at low palmitate levels. This inhibition disappeared when more palmitate was added. Similarly added unlabelled carnitine depressed glycerophosphate acylation, but the effect was relatively small and varied less with the palmitate level. The formation of acylated glycerophosphate was well correlated with the level of acyl-CoA, while production of acylcarnitine still increased with increasing palmitate concentration after the increase in acyl-CoA had ceased (Fig. 1). The results indicate that the affinity of glycerophosphate acylating enzyme towards acyl-CoA is higher than that of ᵗᵉʳ acyl transferase.

ʰᵉ from Fig. 2 and 3, especially carnitine acylation was inhibited by high levels of CoA. The inhibition decreased with increasing palmitate concentrations. Furthermore, the depressing effect of added unlabelled glycerophosphate increased with increasing CoA and decreased with increasing palmitate. The acylation of glycerophosphate was less inhibited by high CoA concentrations. The concentrations of acyl-CoA at different levels of CoA shown in Fig. 4.

acetate-lyase (CoA acetylating) EC 4 1 3 7 85 Units/mg, 24 mg/ml, cat. C 3 60). (−) Carnitine (cat. 5991 d) was purchased from Kock Light Laboratories Ltd, Colnbrook Bucks, England, and Cl reagent (dithiothreitol) from Calbiochem, Los Angeles, Cal. USA. L 3-(^{3}H)-glycerophosphate was pr enzymatically according to Smith & Hubscher (1966), and H methyl labelled (−) carnitine as mad the method of Stokke & Bremer (1970). Scintillation liquid was HEI Unisolve I from Koch laboratories.

Pr paratio f isolated m tochondria Rat li ers homogenized 1 10 ith 10 sucrose with 1 mM (pH 7 4). The homogenate was centrifuged at 800 g for 5 min in order to remove nuclei and cell The mitochondria were sedimented from the supernatant by centrif gation at 8 000 g for 10 min, ones in the same amount of buffer and recentrifuged at 8 000 g for 10 min.

The final pellet was suspended in sucrose solution, so that the concentration of mitochondrial in the suspension was 25 mg/mL. All preparative procedures were performed at 0–2

Incubation procedure The incubation mixture contained in 1 ml 100 μl of the m tochondrial sus (2.5 mg of mitochondrial protein) 25 mM TRIS 5 mM ATP (unless otherwise stated), 2 mM 2 mM KCN 100 mM KCl 15 mg/ml of albumine 1 mM carnitine (unless otherwise stated) 3 glycerophosphate (unless otherwise stated) CoA, palmitate and palmityl-CoA as indicated. T of the mi ture was 7 4 Incubations were carried out at 30° in glass centrifuge tubes w th shaki incubation time was 5 min. Carn tine or glycerophosphate in the incubation mixture ere labe adding small mounts of H labelled compounds w th very high specific radioacti ty making ne changes in the concentrations. Incubations were stopped either by add tion of 3 ml butanol and for 15 s with a whirl mixer o by addit on of 200 μl 70 perchloric acid With the exception of the ments described in Fig 6 a d 7 formation of acylcarnitine and acylated glycerophosphate proceed linear way duri g the incubation.

Assay of acylated carni ne and acylated glycerophosphat The ncubatio mixt re, shaken ml butanol, was supplied with 3 ml water shaken for 15 s with a whirl mixer and centrifuged for in a table centrifuge. The top layer which contained the acylated glycerophosphate nd the carnitine (Daae and Bremer 1970, Bremer 1963), was washed three times w th butanol-saturated Samples were mixed with 10 ml of scintillat on mixture and counted in model 3375 Packard scintillation pectrometer The amou t of acyl ted carnitine or glycerophosphate was calculated by the measured counts per m n te w th the specific acti ty (cpm/nmol) of the carnitine or the phosphate i the incubation mixture after substraction of blank alue (cpm t zero time) nd back By this procedure, carn tin acylatio was registered when carn tine was labelled and glycerophosphate acylation was measured when glycerophosphate was the l belled compound.

Assay of long-chain acyl C A Long-chai acyl-CoA was precipitated w th the proteins when incu were stopped by addition of perchloric acid The precipitate was hydrolyzed w th KOH essentially ac to Tubbs and Garland (1964), and the result ng free CoA was estimated by it catalyzing effect combined phosphate acetyltransferase, citrate synthase and malic dehydrogenase The method is fication of that reported by Michal and Bergmayer (1970) The assay buffer as 50 ml of 0 2 buffer containing 100 mg malate, 50 mg NAD 36 mg acetyl phosphate, 4 mg Cleland reagent (520 Units) malate dehydrogenase, 50 μl (330 Units) phosphat acetyltransferase, and 60 μl (170 citrate synthase at pH 8.0. A sample of 200 μl of the hydrolyzed and neutralized preparation with 2 ml of this assay buffer in a cuvette w th 10 mm lightpath and the linea increase in optical at 340 nm and 30° was registered for 10 min w th a RK C mplet H lger Gilford reaction kinetics photometer Measurements with standard preparations of CoA sho ed that increase in optical of 0.5 per 10 min corresponded to bout 1 mol CoA. The results, after subtraction of the alue at zero incubation time, were considered to represent the level of long-chain acyl-CoA.

General Unless therwise stated, all data represent the means of 6–8 observations Some ariatio one rat to the other was observed but the shape of the figures nd the ariation in the results different varying parameters were reproducible. Bars indicat s tatistical ariations are therefo included in the figures. The results are consequently presented as nanomoles formed. Since the olume incubated suspension of mitochondria in all cases was 1 ml, 1 nmol represents 1 μM concentration. was determined according to Lowry *et al.* (1951).

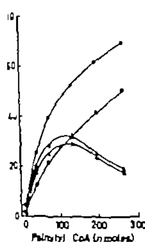


Fig. 6

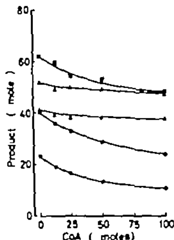


Fig. 7

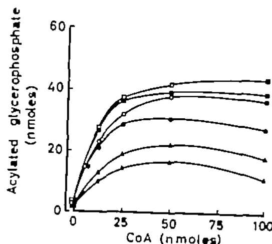
...itine acylation and glycerophosphate acylation at different amounts of added palmityl-CoA. ...e 1: acylcarnitine formed from labelled carnitine in the absence (○) and presence (●) of 250 ...mol, and 2: acylated glycerophosphate formed from labelled glycerophosphate in the absence ...sence (▲) of 250 nmol added palmitate. The amount of added CoA was 50 nmol. ATP was ...n the incubation mixture.

...he inhibitory effect of CoA on acylation and glycerophosphate acylation when palmityl-CoA ...l as substrate. Products are acylcarnitine formed from labelled carnitine (● ○, □) and acylated ...ophosphate formed from labelled glycerophosphate (▲ △). Amounts of added palmityl-CoA are ...(●, ▲), 100 nmol (○,), and 200 nmol (□). The amount of added CoA was 50 nmol. ATP and unlabelled competitive substrates are absent from the incubation mixture.

...he data are not suitable for kinetical analysis, since a significant relative amount of ...l-CoA disappeared during the incubations, i.e. the time course was not linear so ...t values do not *represent initial reaction velocities, especially at low palmityl-CoA* ... Furthermore, since palmityl-CoA is partly bound to albumine (Bremer and Norum ... the concentration of free palmityl-CoA in the incubation medium is not known. ...ntly however, the affinity of the glycerophosphate acylating system towards palmityl-...s higher than that of carnitine acyltransferase. This is on line with data previously ...d by Van Tool (1974).

...s evident from Fig. 7 carnitine acylation was more sensitive than glycerophosphate ...on to inhibition by high levels of CoA. This is in accordance with the results described ... 2 and 3. The degree of inhibition by CoA was highest at the low acyl-CoA level ...), indicating that the inhibition depends on the CoA/acyl-CoA ratio.

...ding a carbohydrate diet to previously fasted rats resulted in decreased carnitine ...ion and increased glycerophosphate acylation in the isolated mitochondria (Table I). ...vident that this effect of dietary treatment was not completed after 4 h of refeeding. ...ate of mitochondrial glycerophosphate acylation was maximal after 10 h of refeeding ...e I). This is probably due to a *relatively high intake ad libitum in the initial refeeding* ...d.



F g. 3

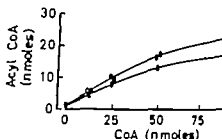


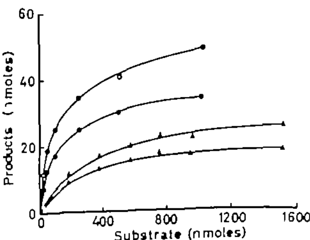
Fig. 4

Fig. 3 Glycerophosphate acylation t different amounts of added CoA Open symbols indicate the ab and filled symbols the presence of unlabelled carnitine. The amounts of added palmitate ere 330 (□ ■), 220 mol (○ ●) and 110 nmol (△ ▲). No palmityl-CoA was added.

Fig. 4 The level of acyl-CoA at d fferent amounts of added CoA. The levels of acyl-CoA were form the presence of carnitine (○), glycerophosphat (⊖) or both (●). The amount of added palmitate a nmol.

The results with varying concentrations of H labelled carnitine or glycerophosph at constant specific radioactivities, are described in Fig. 5 The hyperbolic curves corresp to K_m(carnitine) = 0 12 mM and K_m(glycerophosphate) = 0 31 mM The degree of depres by the competitive substrate is about constant at varying concentrations of labelled s strates.

The rates of carnitine acylation and glycerophosphate acylation when varying amou of palmityl-CoA was added to the incubation system are shown in Fig. 6. In this experime no ATP was added, so that no synthesis of palmityl-CoA took place. Still, addition palmitate stimulated carnitine acylation, while there was only little effect on glyceroph phate acylation Glycerophosphate acylation was inhibited by high concentrations of ac



F g. 5 Carnitine acylation and glyce phosphate acylation at different amou of added labelled carnitine or label glycerophosphate. Products are l ac carnitine formed from labelled carnit in the bsence (○) nd presence (f unlabelled glycerophosphate, and acylated glycerophosphat from labell glycerophosphate in the bsence (and presence (▲) of unlabelled carnit The pecific adioacti ities of adde labelled carnitine and glycerophospha were kept constant. The amount of adde palmitate was 220 mol, and the amou of added C A was 50 mol. No palmity CoA as added.

logical importance. Several reports indicate that this ratio does not change appreci- n rats are fasted (Pearson and Tubbs 1967 Tubbs and Garland 1964 Kondrop ... 1973, Bortz and Lynen 1963, Greenbaum *et al.* 1971). However if free fatty l acyl-CoA compete for binding sites, a lowering of the fatty acid level due to carbo- feeding may permit protein binding of acyl-CoA, thus increasing the CoA/acyl- io available to the different enzymes.

...pid inhibitory effect of glucose on fatty acid oxidation (Lossow and Chaikoff 1955) mediated through increased insulin secretion, causing decreased levels of fatty the blood and liver tissue. If the data in fig. 1 are relevant in this context, a fall vel of fatty acids would bring about a relatively proportional decrease in the rate of e acylation, and thereby a low level of acylcarnitine. If the tissue level of long- cylcarnitines and fatty acid oxidation are correlated with each other as suggested r 1967 Pearson and Tubbs 1967), the results would be decreased oxidation of fatty ...hus, the kinetical characteristics of the system could be a factor in the mechanism glucose-effect. The dietary adaptation of the enzymic reactions is relatively slow ...), and is therefore not the mechanism of the rapid inhibition of fatty acid oxidation ...ose, which is established within one hour after glucose feeding (Lossow and Chaikoff ...lohmer 1967 Jansen *et al.* 1966). However the dietary adaptation possibly leads to er decrease in fatty acid oxidation after the rapid effect of glucose is established.

...rk was aided by grants from Anders Jahres medical foundation and from Nordic Insulin Fund. ...hd technical assistance of A. Trana is gratefully acknowledged. The author is indepted to Professor ...r for helpful suggestions and criticism.

References

...and H. W. DAAE, Fatty acid activation and acyl transfer in organs from rats in different nutritional ... *Biochim. biophys. Acta* (Amst.) 1971 *239* 208–216.
... W. M. and F. LYNEN, Elevation of long-chain acyl-CoA derivatives in livers of fasted rats. *Biochem.* ...63 *339*, 77–82.
..., J., Carnitine in intermediary metabolism, the biosynthesis of palmitylcarnitine by cell subfractions. ...*d. Chem.* 1963, *238*, 2774–2779
..., J. and K. R. NORUM, Palmityl-CoA: Carnitine O-palmityltransferase in the mitochondrial oxida... of palmityl-CoA. *Eur. J. Biochem.* 1967 *1* 427–433
..., T. Tissue levels of activated fatty acids (acylcarnitines) and the regulation of fatty acids (acyl-...nitines) and the regulation of fatty acid metabolism. *Biochim. biophys. Acta* (Amst.) 1967 *144* 259–
...
... L. N. W. and J. BREMER, The acylation of glycerophosphate in rat liver. A new assay procedure for ...erophosphate acylation, studies on its subcellular and submitochondrial localization and determina- ... of the reaction products. *Biochim. biophys. Acta* (Amst.) 1970, *210*, 92–104.
...AN, M., J. BREMER and K. R. NORUM, Long-chain acyl-CoA synthase in rat liver. A new assay pro-...ure for the enzyme, and studies on its intracellular localization. *Biochim. biophys. Acta* (Amst.) ...7 *132* 493–502.
...MAUM, A. L., K. A. GUMAA and P. McLEAN, The distribution of hepatic metabolites and the control ...the pathways of carbohydrate metabolism in animals of different dietary and hormonal status. *Arch. ...chem.* 1971, *143*, 617–663.
..., O. R., M. E. ZANETTI and C. F. HUTCHINSON, Studies on lipogenesis in vivo. Effects of starvation ...e-feeding, and studies on cholesterol synthesis. *Biochem. J.* 1966. *99* 333–340.
...ELY, L. and N. GRUNNET, The effect of acute and prolonged ethanol treatment on the contents of ...enzyme A, carnitine and their derivatives in rat liver. *Biochem. J.* 1973, *132* 373–379

Discussion

It has been suggested that changes in the relative activities of the glycerophosphate acylating and carnitine acylating enzymes depending on diet may regulate the distribution of fatty acids between oxidation and esterification in the liver (Aas and Daae 1971). The moderate changes in enzyme activities, and also in the relative high activity of the fatty acid activating enzyme in relation to the acyl-CoA utilizing reactions, gave rather dubious support to this hypothesis. However the results presented here (Table 1) show that the kinetic properties of the enzymes involved combined with changes in the concentration of fatty acids may very well lead to pronounced changes in the fatty acid distribution. Thus, the rate of carnitine acylation at 880 μM palmitate in mitochondria from fasted rats was 5-6 times higher than that at 220 μM palmitate in mitochondria from fasted/refed rats (Table 1).

It may be objected that only isolated mitochondria have been used in this study while in the intact cell fatty acid activation and glycerophosphate acylation take place both in the endoplasmatic reticulum and in the mitochondria (Kornberg and Pricer 1953, Farstad *et al* 1967 Daae and Bremer 1970) However with a more complicated system, *e.g.* the complete particulate fraction of a liver homogenate, it proved much more difficult to obtain reproducible results. We have therefore performed these primary studies with isolated mitochondria which gave reproducible results. Rat liver mitochondria also contain an appreciable fraction of the total capacities for fatty acid activation and glycerophosphate acylation in that tissue (Farstad *et al* 1967 Daae and Bremer 1970). Isolated mitochondria therefore presumably represent a good model system for these studies.

The small response of glycerophosphate acylation to variations in palmitate concentrations (Fig. 1 and Fig. 6) is most likely explained by a relatively high affinity of the glycerophosphate acyltransferase towards acyl-CoA It has been previously reported that the K (acyl-CoA) of carnitine acyltransferase is higher than that of glycerophosphate acyltransferase (Van Tool 1974). Also the data presented in Fig. 1 and Fig. 6 indicate a difference in the apparent K_m (acyl-CoA) values of the two enzymes. It should be stressed that the difference is observed under identical conditions, and is therefore valid even if it is close to meaningless to measure K_m for a lipid substrate with low solubility and a tendency of binding to proteins and other lipids.

The difference in affinity for the relevant enzymes may be of importance when seen in relation to the binding of acyl-CoA to albumine and presumably other proteins. The stimulation of acylcarnitine formation by palmitate (Fig. 6) is probably due to competition with acyl-CoA for binding sites on proteins, resulting in increased free acyl-CoA and thereby increased acylcarnitine formation Competition between palmitate and acyl-CoA will lead to a more complete binding of acyl-CoA at low levels of the fatty acid and make carnitine acylation more susceptible towards the competitive action of glycerophosphate (Fig. 1). Similar kinetics as those presented here may be valid in the intact liver cell if some cytoplasmic or structural protein binds fatty acids and acyl-CoA A fatty acid binding protein has been isolated from animal tissues (Mishkin *et al* 1972, Mishkin and Turcotte 1974).

The results presented in Fig. 7 show that carnitine acylation is inhibited more by free CoA (a high CoA/acyl-CoA ratio) than is the acylation of glycerophosphate. This may be

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om the Laboratory for the Theory of Gymnastics, University of Copenhagen, Denmark

Thermoregulation during Static Work with the Legs

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Abstract

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and static work with the legs, i.e. holding weight of about 10 of maximal isometric strength for was compared to dynamic exercise on bicycle ergometer causing the same rate of heat production. In static work the subjective feeling of exertion was very high and the effort maximal. The pronounced heart rate and blood pressure and typical flush of the face and chest (flush areas) indicated sympathetic tonus. Plasma catecholamine levels were 1.3 times higher (significant at the $P < 0.05$ after static work than after dynamic work. Although the sustained static work was nearly maximal, of increase in sweating and the change in core temperature during work were not different from sponses to dynamic work.

namic work deep body temperature is increased in proportion to the relative load on subject (% of max $\dot{V}_{O_2}$) and proportional to the subjective feeling of exertion. In contrast, static effort, i.e. sustained isometric muscle contraction, is felt very exhausting, and t rate and blood pressure reach high values, in spite of the low levels of oxygen uptake tained during this activity (Lind *et al.* 1964). This may be explained as resulting from creased sympathetic tonus.

t has been reported that sweat glands are sensitive to adrenaline and noradrenaline tions (Allen and Roddie 1972). Further Robertshaw and others (1973) demonstrated t in sympathectomized macaques (*Macaca speciosa*) sweating during treadmill exercise was endent on the increased secretion of adrenaline associated with exercise. In the present dy the aim was to compare static and dynamic work to see if the much higher subjective ling of effort in static work had a specific effect on the thermoregulatory reactions to work.

Methods

subjects were three well trained students. Their data are presented in Table I. For static work the sub- t sat on a seat with a low back-rest with his legs slightly bent (120° between upper and lower leg). He sed his feet against a transverse bar supplied with footrests and lifted the suspended weights through system. He held the load in a fixed position as long as possible but at least 20 min. or dynamic work he pedalled a Krogh bicycle ergometer in which the saddle was replaced by an arm- ir. In this way the position was nearly the same in the two kinds of work.

LOSSOW W J and I L. CHAIKOFF Carbohydrate sparing of fatty acid oxidation. *Arch. Biochem.* 19 *57* 23–40.

LOWRY O H N J ROSEBROUGH, A. L. FARR and R. J RANDALL, Protein measurement with the phen reagent. *J biol. Chem* 1951 *193* 265–275

MAYES, P A. Studies on the major pathways of lipid metabolism using the perfused liver In *Adip tissue regulation and metabolic functions* Ed B. Jeanrenaud and D Hepp, Georg Thieme Verlag Stuttg 1970. 186–195.

MCGARRY J D J M. MEIER and D W FOSTER, The effects of starvation and refeeding on carbohydr and lipid metabolism *in vivo* and in the perfused rat liver The relationship between fatty acid oxidat and esterification in the regulation of ketogenesis. *J biol Chem.* 1973 *248* 270–278.

MICHAL, G and H U BERGMAYER, Bestimmung von CoA SH mit phosphotransacetylase. In *Method der enzymatischen analyse Band II* Ed H U Bergmayer Verlag Chemie Weinheim/Bergstr 1970. 19 1905.

MISHKIN, S. and R. TURCOTTE, The binding of long chain fatty acid CoA to Z, a cytoplasmic prot present in liver and other tissues of the rat. *Biochem. biophys. Res. Commun.* 1974. *57* 918–926.

MISHKIN S., L. STEIN, Z. GATMAITAN and I M. ARIAS, The binding of fatty acids to cytoplasmic protei Binding to Z protein in liver and other tissues of the rat *Biochem. biophys. Res. Commun.* 1972. 997 1003

NORUM, K R Activation of palmityl CoA carnitine palmityltransferase in livers from fasted, fat-f or diabetic rats. *Biochim. biophys Acta* (Amst.) 1965 *98* 652–654.

NORUM K. R. and J BREMER, The localization of acyl-coenzyme A-carnitine acyltransferase in rat li cells. *J biol. Chem* 1967 *242* 407–411

PEARSON, D J and P K. TUBBS, Carnitine and derivatives in rat tissues. *Biochem. J* 1967 *105* 953–9

RANDLE, P J C. N HALES, P B. GARLAND and E. A NEWSHOLME, The glucose fatty-acid cycle, its r in insulin sensitivity and the metabolic disturbances of diabetes mell tus. *Lancet* 1963 *1* 785–792.

SHIPP J C., Interrelation between carbohydrate and fatty acid metabolism of isolated perfused rat hea *Metabolism* 1964 *13* 852–860.

SMITH, M E. and G HÜBSCHER, The biosynthesis of glycerides by mitochondria from rat liver the requi ment for a soluble protein. *Biochem. J* 1966. *101* 308–316.

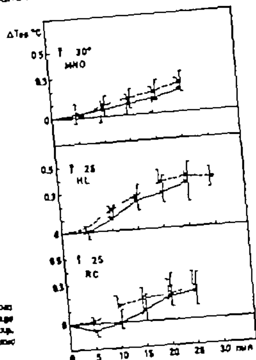
STOKKE, O and J BREMER, A simple method fo preparation of methyllabelled ( ) carnitine. *Bioch biophys. Act* (Amst.) 1970. *218* 552 554

TUBBS, P K. and P B GARLAND, Vari tions in tissue contents of coenzyme-A thio esters and poss metabolic implications. *Biochem. J* 1964. *93* 550–557

VAN TOOL, A The effect of fasting on the acylation of carnitine and glycerophosphate rat liver s cellular fractions. *Biochim. biophys Acta* (Amst.) 1974. *357* 14–23

VAVRECKA, M M. P MITCHELL and G HÜBSCHER, The effect of starvation on the incorporation of palmit into glycerides and phospholipids n rat liver homogenates. *Biochem. J* 1969 *155* 139–145.

ZBOROWSKI, J and L. WOJTCZAK, Phospholipid synthesis in rat li er mitochondria. *Biochem. biophys. A* (Amst.) 1969 *187* 73–84



1. Change in deep oesophageal temperature from resting levels plotted against work time. Average and range shown for 3 subj. Number of exp. Table I. Static work (●) with range, dynamic k (...) with range.

Results

The change in core temperature and the change in weight due to sweating in the first 20–30 min of dynamic and static work are compared in Fig. 1 and 2. For all subjects there is a ... order of the values, but the time course for weight loss and T_{es} change appear to be the same in the two kinds of "work.

The average values obtained after 20 min of static and dynamic work for H V_{O_2}, HR, T_{re}, T_{es}, $\bar{T}$ and weight loss are plotted against each other in Fig. 3.

One parameter that is strikingly different in the 2 conditions is the heart rate, which is [illegible] beats (41%) higher in subject RC, 28 beats (30%) higher in subject HL, and 13 beats ([illegible]%) higher in subject MNO during static work.

Table II.

Blood pressure

Subject	Rest	Dyn.	Stat.
	sitting	(20 min)	(20 min)
RC	132/92	144/84	153/105
	±3	±1	±2
MNO	129/85	135/91	178/118
	±11	±5	±6

TABLE I

Subject	Height cm	Weight kg	Max V_{O_2}	Max HR	Max isom. strength legs kp	Work	
						Dynamic kpm/min	Static kp
HL	189	80	4 45	183	670	360 n = 6	67 n 6
RC	180	89	4 71	189	840	450–520 n = 5	100 n 6
MNO	186	80	5.19	198	607	198 n = 5	91 93 6 n 5

Both work machines could be suspended in a Krogh balance. Weight loss during the experiments co therefore be measured continuously (Nielsen and Nielsen 1965).

The dynamic work intensity was chosen so as to give the same rate of heat production, *H* as the st work *i.e.* oxygen consumption con verted to calories, *M* minus the external work in calories, *W* dur dynamic work, equals oxygen consumption (calories) in static work, where no external work is d $(M - H)$. This was very nearly obtained as seen in F g. 3 The higher oxygen uptake during dynamic w is thus explained. The V_{O_2} fo the chosen wo k-load decreased with training n some subjects. It was the fore sometimes necessary to increase the load to keep *H* at the initial value.

The respiratory gas exchange was determined by the Douglas bag method. Dried air samples analyzed on a paramagnetic oxygen analyser (Servomex OA 184) and on an infrared CO_2 analyser (Bo man LB 1).

Deep esophageal temperature (T_{oe}) was measured with a thermocouple inserted through the nose placed i the lower esophagus just above the diaphragm. The placing was controlled in each subject x-ray photography (Nielsen and Nielsen 196.). The temperature was recorded on a Kipp Microgr BD 1 The skin temperature (T_s) was measured at 15 locations with a thermocouple (Nielsen and Niel 1965). The average skin temperature ($\bar{T}_s$) was computed by weighi g each measurement according to size of the corresponding skin area (Hardy and Dubois 1938). In two of the subjects the muscle temperat (T_m) was measured w th needle probe the vastus lateralis 2½ cm depth bef re and after ork. T measurements of T_{oe} and T_m were accurat to within $\pm 0.0.5°C$, T to within $\pm 0.05°C$. Heart rate w counted from an ECG registration.

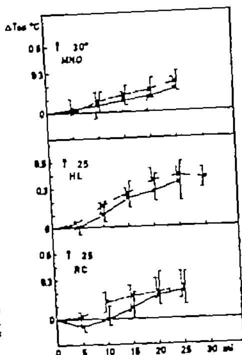
In some experiments on RC, and in all on MNO blood pressure was measured by the usculat method over the brachial artery In HL nd RC 5 ml blood sample was taken from the cubital v 2 min after work had ended and analyzed for lactat by an enzyme method (Scholz *et al.* 1959). In subj MNO (dynamic or static) work was stopped at exactly 25 min, and 40 ml venous blood from the cubi vein was taken into pre-cooled glass containing 50 mg ascorbic acid and 50 mg EDTA. The blood sampl were centrifuged immediately after at 4 000 rev/min for 10 min t 0° n cooling centrifuge. The plasm was removed and stored for later analysis for oradrenaline (NA) and adrenaline (A) by the meth described by Engelman *et al.* 1968 and 1970.

The experiments took place in climatic chamber at 25 ±½ C (subj. HL and RC), or t 30°±½ C (su MNO). Humidity was kept below 75 RH

Procedure

The subject arrived fasting. He rested for 45 min in the ergometer seat. The resting T_{oe} and $\bar{T}$ ere th recorded and HR counted. Resting metabolic rat was measured n some experiments. Weight loss duri rest was registered over a 10-min period.

He then started to work, either pedalling 60 rev/min t low work-load (198–540 kpm/min), giving rate of heat production equal to that he could maintain f 25 m in static effort — or lifting and holdi the weight in the required position for static effort Heart rate was counted every 5 min. $\bar{T}$ as measure at approx. 8, 12 and 18 min. Douglas bags were f lled t 10 nd 16 min. When measured, blood pressu was taken at rest, at 9 and at 19 min work.



...hange in deep esophageal temperature from ...ine levels plotted against work time. Average ...d range shown for 3 subj. Number of exp. ...le I. Static work (●|) with range, dynamic ...) with range.

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...LE II.

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...bject	Rest	Dyn.	Stat.
...C	sitting	(20 min)	(20 min)
	132/91	144/86	153/105
	2	1	−2
MNO	129/83	135/91	178/118
	11	5	−6

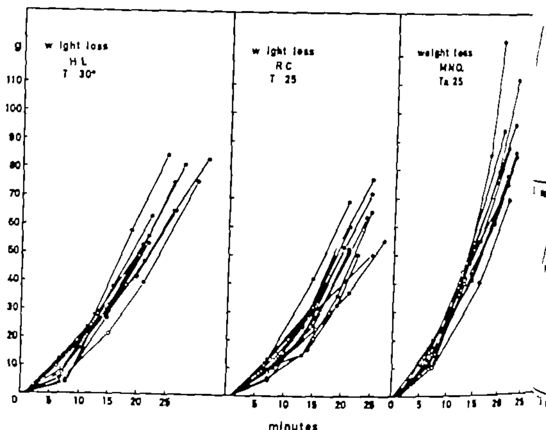


Fig. 2. Total weight loss plotted gainst wo k time (3 subj.) Static work ● dynamic work O

Also blood pressures where measured were higher during static work Table II. Blo lactate after work was 1 6 m mol/l after static, 0,6 m mol/l after dynamic work. When at exhaustion in the static exercise, the subjects showed a very prominent flush of the face a chest (flush areas")

The plasma catecholamine levels in plasma samples taken after 25 min work were high after static than after dynamic work at the P 0.05 level The values are shown in Table II

Discussion

The experiments showed that the maximal effort and the very pronounced subjective feeli of exertion during static work had no specific effect on the activation of sweating and the elevation of body temperature.

TABLE III Plasma values g/ml of noradrenali e (NA) and adrenaline (A) after 25 min of static a dynamic work (subj MNO). Normal alues (30). NA 0.36 ± 16. A 0.10 ± 0 05.

	NA $\bar{x}$ S.D	A $\bar{x}$ S.D
static work n = 6	0.64 ± 0.091	0.30 ± 0.050
dynamic work n = 6	0.50 ± 0.050	0 23 ± 0.045

Static work values are significant different from dynamic work alues, 0.01 < P < 0.05 (T test).

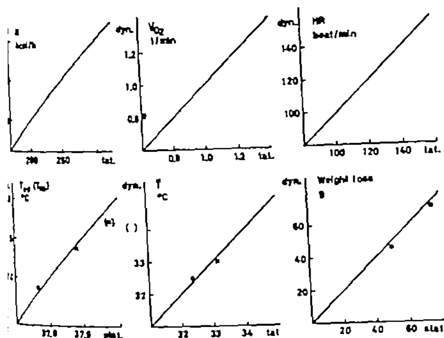


Fig. 3 Comparisons of average values of heat production H, oxygen consumption V_{O_2}, heart rate HR, esophageal temperature at 20 min of work T_{es} (muscle temperature after work [illegible] brackets (T_m)), [illegible] skin temperature T_s, and weight loss at 20 min during static and dynamic work. Subj. RC ●, subj. HL [illegible] ○.

In prolonged exercise, sweating usually is correlated with rate of heat production (B. Nielsen 1966, 1968, 1969) even when neuromuscular activities are quite different (positive compared to negative work,—arm work to leg work and—as here—static compared to dynamic work). However at the onset of dynamic work (within the first min) the rate of increase in sweating has been found to depend on the intensity of exercise (Beaumont and Bullard 1963).

This reaction seemed to depend on neural stimuli of central nervous or reflex origin, since no thermal effect on central thermoreceptors could have had time to act.

Robertshaw *et al.* 1973 found that in macaques adrenal denervation decreased sweating during treadmill exercise by approx. 50%, while adrenaline infusion restored sweating to normal levels. In man the sympathetic nerve activity and the plasma level of circulating catecholamines increase during work proportional with the relative load. (Häggendal, Hartley and Saltin 1970). It is possible that the skin circulation goes down due to the increasing constrictor tone and thus becomes related to the relative load. This again causes the core temperature to increase in proportion to the relative load (Saltin and Hermansen 1966). The latter follows from the equation

$\dot{Q}$ – skin blood flow

c – heat capacity of blood

$\bar{T}_{art}$ and $\bar{T}_V$ – average temperature of arterial and venous blood from skin.

Kozlowski *et al* (1974) found a significant increase in both NA and A with static (handgrip 30 of max. handgrip) and an increase that was about 1½ times greater than obtained after heavy dynamic work with the legs. In the present static leg work NA twice and A three times higher than values determined by the same method on resting subjects, and both NA and A levels were 1 3 times higher (significant at $P < 0.05$) after static than after dynamic work

However the present experiments do not support the hypothesis that a high sympa tone should force the core temperature upwards. In spite of signs of sympathetic and ad activity (high heart rate, blood pressure and higher plasma NA and A) the core temper during static work increased in the same manner as in the dynamic work, nor did the crease in sweating differ in the two types of work

I w t to express my grat tude to Dr Frans Fog-Moller Dept of Clinical Chemistry F ederiksberg tal, who made the catecholamine analyses.

References

ALLEN, J A and I C. RODDIE, The role of circulating catecholamines n sweat production in m *Physiol* (Lond.). 1972. 227 801–814

BEAUMONT W van and R. W BULLARD, Sweating: its rapid response t muscular work. *Science* 141 643–646.

ENGELMAN, K. and B. PORTNOY A sensit e double-isotope derivati assay for norepinephrine epinephrine C c *lat Res* 1970, 26, 53–57

ENGELMAN, K., B PORTNOY and W LOVENBERG, Sensitive and specific double-isotope derivative meth determination of catecholam nes in biological spec mens. *Amer J med. Sci.* 1968. 255 259 268.

HARDY J D and E F DUBOIS, The technic of measuring radiation and con ection. *J Nutr* 19 461–475

HÄGGENDAL, J L H H RILEY and B. SALTIN Arterial noradrenaline concent ation during exer relation to the relative work levels. *Scand J lin Lab Invest* 1970, 26 337 34

KOZLOWSKI S. Z. BRZEZINSKA K N ZAR, W KOWAL K and M FRANCZYK Plasma catechol a during sustained isometric exercise *Clin Sci Mol. M d* 1973 45 723 731

LIND, A. R S. H TAYLOR, S. H HUMPHREYS, B. M KENNE Y and K W DONALD, The circulatory of sustained oluntary muscle contraction *Clin. Sc* 1964 27 229– 44

NIELSEN B R gulation of Body Temperatu e nd Heat Dissipation at Different Levels of Energy Heat Production in Man. *Act physiol scand.* 1966 68 215–227

NIELSEN, B., Thermoregulatory Responses to Arm Work Leg Work and Intermittent Leg Work. *physiol. scand.* 1968. 72 25–32.

NIELSEN, B Thermoregulation I Rest and Exercise *Act ph siol. scand.* 1969 Suppl 323.

NIELSEN, B. and M NIELSEN, Body t mperature during work at different envir nmental tempera *Acta physiol scand.* 196. 56. 120–129

NIELSEN B and M NIELSEN, On the regulation of weat secretion n exercise. *A ta ph siol scand.* 64 314 322.

ROBERTSHAW D R. TAYLOR and L M M ZZIA, Sweat g primates secretion by adrenal me during exercise. *Amer J Physiol.* 1973 224 678 681

SALTIN, B. and L. HERMANSEN, Esophageal, rectal nd muscle temperature d ring exercise. *J appl Ph* 1966. 21 1757 1762.

SCHOLZ, R., H. SCHMITZ, T BUECHER and J O LAMPE Über die Wirkung on Ny tat n auf Bäcker *Biochem. Z* 1959 331 71–86.

Acta physiol. scand. 1975. 95. 463–469

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Vascular Resistance in Peripheral Blood Vessels at Normotension and at Local Orthostatic Hypertension in Healthy Humans

By

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Abstract

PAASKE, W. P. and O. HENRIKSEN. *Vascular resistance in peripheral blood vessels at normotension and at local orthostatic hypertension in healthy humans.* Acta physiol. scand. 1975. 95. 463–469.

The effect of changes in orthostatic pressure on vascular resistance in subcutaneous adipose tissue was studied in the forearm at normal tension and in the leg at local orthostatic hypertension in three normal subjects. Blood flow in subcutaneous tissue was measured by the local xenon-133 washout technique. Changes in orthostatic pressure were achieved by postural changes of the extremity. At levels above the heart where venous pressure is constant, there is an autoregulation of blood flow over a wider arterial blood pressure range in leg than in forearm. When transmural pressure increased 25 mm Hg or more vascular resistance increased about 50 per cent in the dependent forearm and 300 per cent in the dependent leg. The effect of ischemia on vascular resistance was investigated in cutaneous tissue of hand and foot by means of the ^{99m}Tc-Antipyrine initial slope technique. Vascular resistance after maximum dilatation achieved by 20, 30, and 35 min of ischemia was less in vessels of the hand than in vessels of the foot. Thus, adaptive structural changes of vessels subjected to increased blood pressure are present in cutaneous and subcutaneous adipose tissues in normal subjects.

Hypertensive patients have an increased vasoconstrictor response, augmented constriction after stimulation by noradrenaline, and enhanced resistance after maximum vascular dilatation (Folkow *et al.* 1958, Conway 1963, Sivertsson 1970). It has previously been shown that vascular resistance in cutaneous and subcutaneous adipose tissues of the dependent arm increased some 50 per cent compared with resistance at level of the heart (Henriksen *et al.* 1973, Henriksen, Nielsen, and Paaske 1973).

When a person is upright vessels of the legs are subjected to higher hydrostatic pressures than are vessels of the arm. Thus the effect of changes in orthostatic pressure and vascular resistance during maximum dilatation obtained in areas subjected to local orthostatic hypertension (leg) can be compared to vascular reactions of normotensive areas (arm).

The present study was performed to investigate whether adaptive structural changes of vessels subjected to orthostatic hypertension were present in normal subjects.

Experimental procedure

The experiments were carried out on three healthy subjects. Room temperat re was kept constant at

I Orthostatic pressure changes

(A) *Subcutaneous adipose tissue of forearm.* 0.2–0.4 ml xenon-133 with a concentration of 5 mCi isotonic saline was injected ntracutaneously 5 cm proximal to the wrist of the forearm. About 90 mi the i jection the xenon-133 is exclusively located in subcutaneous tissue (Sejrsen 1971). This techniq used to a oid jection trauma to the subcutaneous tissue. After 90 min the measurements re The subject was placed n a sitting position and the labelled area was placed at reference level, the ju notch.

A NaI (Tl) scintillation detector was connected to a universal printing gamma-spectrometer (Selo Denmark) w th an energy window of cceptance adjusted symmetrically round the 0.081 MeV photo of xenon-133 A trial consisted of three periods of measurements, each of about 5 min duration, first the labelled area at reference level, then dislocated passively to a test level, and finally again th the at reference level.

(B) *Subcutaneous adipose tissue f leg* Xenon-133 was injected intracutaneously 5 cm proximal t lateral malleolus. The measurements were performed as described abo e, the reference level bein midax llary line as the subject was now in supine position.

II Postischemic hyperemia in cutaneous tissue

Pressures of ±80 mm Hg were applied by cuffs placed just proximal to wrists and malleoli of one su placed supine position. 0 05 ml (10–20 μCi) ^{131}I Antipyrine was injected intracutaneously in the bac each hand and foot 3 cm distal to the cuff Skin above each deposit was heated by lamps. After 5, 10 20, 30, and 35 min the cuff were deflated Washout of ^{131}I was simultaneously recorded by NaI scintillation detectors connected to gamma-spectrometers adjusted symmetrically around the 0 364 photopeak of ^{131}I

The influence of 35 min f ischemia pon kin temperature was studied separately Arterial blood p sure was measured w th sphygmomanometer on the arm. Venous blood pressures ere measured venopuncture of a superficial vein of the forearm.

Calculations

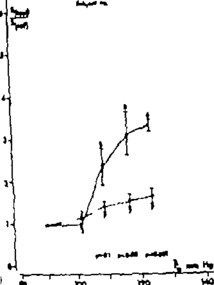
Mean perfusio coefficient, $\bar{f}$ n subcutaneous adipose tissue was calculated from the Kety equat $\bar{f} = k \cdot \lambda \cdot 100$ (ml/100 g min) where k denotes the washout rat constant (min) and λ the blood to adip tissue partition coefficient (ml/g) (Kety 1951) A λ alue of 10 ml/g was used (Sejrsen 1971 Nielsen 197

Maximum hyperemic blood flow n cutaneous tissue was estimated from the ashout rate constant the initial monoexponential part of the ^{131}I Antipyrine washout curve. The product of maximum rate constant times duration of hyperemia (k_{max} t) was calculated (Lindbjerg 1966, Nielsen and Sej 1972). Arterial mean pressure at reference level ($\bar{P}_{a,ref}$) was calculated and $\bar{P}_{a,test}$ alues ere estimat Venous pressure was measured in forearm and calculated fo leg levels. Fractional change of blood fl and relative change in total ascular resistance were calculated for each experiment.

Results

Results obtained during lowering the extremity are shown in Fig. 1 1–3 expts. were pe formed. Relative resistance remained constant when arterial blood pressure was increase from about 90 mm Hg to about 105 mm Hg in both arm and leg. When arterial pressure wa further elevated relative resistance increased about 50 per cent in arm and 300 per cent in leg The difference in vasoconstrictor response was significant.

Results obtained during elevation of the extremity are shown in Fig. 2. 58 expts. wer performed. When arterial blood pressure was lowered from 90 mm Hg to about 60 mm H (*i.e.* venous pressure is constant) blood flow remained constant in both arm and leg. Belo



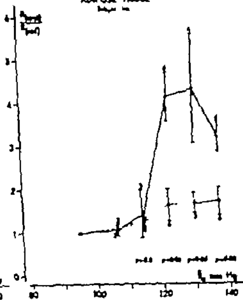
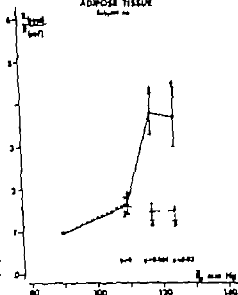


Fig. 1 a, b, and Relative change in total vascular resistance (R_{heat}/R_{pref}) as function of mean arterial blood pressure ($\bar{P}_a$). Open circles and the broken line refer to experiments performed on the distal part of forearm, filled circles and the full line to experiments on the distal part of crus. p-values indicate significance level between response obtained in forearm and crus (randomization test for two independent samples). The horizontal bars signify 1 S.E.



an arterial blood pressure of 60 mm Hg blood flow decreased in proportion to the reduction of arterial perfusion pressure in both arm and leg.

Maximum washout rate constants obtained after different duration of ischemia are shown in Fig. 3. 10 expts. were performed on one subject. Maximum washout rate constant did not increase any further when duration of ischemia was extended from 20 min to 35 min.

Experimental procedure

The experiments were carried out on three healthy subjects. Room temperature was kept constant at °(

I Orthostatic pressure changes

(A) *Subcutaneous adipo e tissue of f earm.* 0.2–0.4 ml xenon-133 w th a concentration of 5 mCi/ml isotonic saline was injected intracutaneously 5 cm proximal to the wrist of the forearm. About 90 min af the injection the xenon-133 is exclusively located in subcutaneous tissue (Sejrsen 1971). This technique used to oid injection trauma to the subcutaneous tissue. After 90 min the measurements ere start The subject was placed in a sitting position and the labelled area was placed at reference level, the jug notch.

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(B) *Subcutaneous adipose tissue f leg* Xenon-133 was injected ntracutaneously 5 cm proximal to t lateral malleolus. The measurements were performed as described above, the reference level being t midaxillary line as the subject was now in supine position.

II Postischemic hyperemia in cutaneous tissue

Pressures of 280 mm Hg were pplied by cuffs placed just proximal t wrists and malleoli of one subje placed in supine position. 0 05 ml (10–20 μCi) ^{131}I Antipyrine was injected intracutaneously in the back each hand and foot 3 cm distal to the cuffs. Skin abo e each deposit was heated by lamps. After 5, 10, 1 20, 30 and 35 min the cuffs were deflated. Washout of ^{131}I was simultaneously recorded by NaI (T scint llation detectors connected to gamma-spectrometers adjusted symmetrically around the 0.364 Me photopeak of ^{131}I

The influence of 35 min of ischemia upon skin temperature was studied separately Arterial blood pre sure was measured with a sphygmomanometer on the arm. Venous blood pressures were measured b venopuncture of a superficial vein of the forearm.

Calculations

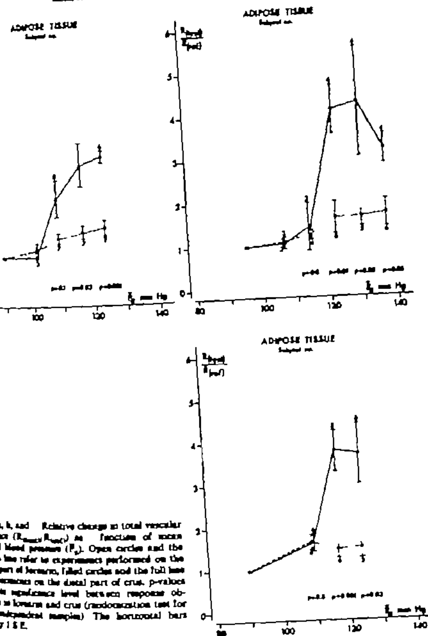
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II *Postischemic hyperemia in cutaneous tissue*

Pressures of 280 mm Hg were applied by cuffs placed just pro imal to wrists and malleoli of one subje *placed in supine position. 0 05 ml (10–20 μC) ^{131} I Antipyrine was njected intracutaneously* in the back each hand and foot 3 cm distal to the cuffs. Skin abov each deposit was heated by lamps. After 5, 10, 1 20, 30, and 35 min the cuffs were deflated Washout of ^{131} I was simultaneously recorded by NaI (T scintillation detectors connected to gamma-spectrometers adjusted symmetrically around the 0.364 Me photopeak of ^{131} I

The influence of 35 min of ischemia upon skin temperature was studied separately Arterial blood pre sure was measured with a sphygmomanometer on the arm. Venous blood pressures ere measured venopuncture of a superficial vein of the forearm.

Calculations

Mean perfusion coefficient, $\bar{f}$ n subcutaneous adipose tissue was calculated from the Kety equation $\bar{f} = k\ \lambda\ 100$ (ml/100 g min) where k denotes the washout rate constant (mi) and λ the blood t adipo *tissue partition coefficient (ml/g) (Kety 1951). A λ value of 10 ml/g was used* (Sejrsen 1971 Nielsen 1972

Maximum hyperemic blood flow in cutaneous tissue was estimated from the washout rate constant the initial monoexponential part of the ^{131}I Ant pyrine washout curve. The product of maximum ashot rate constant times duration of hyperemia (k_{max} t) was calculated (Lindbjerg 1966, Nielsen and Sejrs 1972). *Arterial mean pressur at reference level* ($\bar{P}_{a(ref)}$) was calculated and $\bar{P}_{a(test)}$ alues were estimate Venous pressure was measured n f rearm and calculated fo leg levels Fractional change of blood flo and relative change in total vascular resistance were calculated for each experiment

Results

Results obtained during lowering the extremity are shown in Fig. 1 123 expts. were per formed. Relative resistance remained constant when arterial blood pressure was increase from about 90 mm Hg to about 105 mm Hg in both arm and leg. When arterial pressure wa further elevated relative resistance increased *about 50 per cent in arm and* 300 per cent in leg The difference in vasoconstrictor response was significant

Results obtained during *elevation of the extremity are shown in* Fig. 2. 58 expts. wer formed. When arterial blood pressure was lowered from 90 mm Hg to about 60 mm H

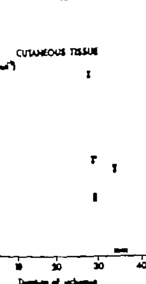


Fig. 3

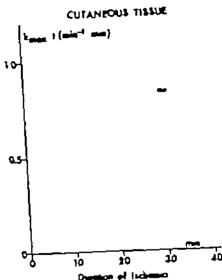


Fig. 4

...he maximum washout rate constant (k_{max}) of ^{131}I-Antipyrine washout from cutaneous tissue as ...of duration of ischemia. Open circles denote hand, filled circles foot. Each square represents the ... in k_{max} values between hand and foot in one experiment.

... The product of maximum washout rate constant times duration of hyperemia (k_{max} t) as function ...on of ischemia. Open circles denote hand, filled circles foot. The Spearman rank correlation coef... r_s 0.99 and 0.91 for hand and foot (p 0.01, one-sided significance level, for both regions).

...ation coefficient, r_s, was 0.99 ($p < 0.01$) for hand and 0.91 ($p < 0.01$) for foot (one sided ...ance level). k_{max} t of hand and foot did not differ (randomization test for paired ...vations, p 0.9).

Discussion

...influence of orthostatic pressure changes on blood flow in subcutaneous adipose tissue ...forearm and leg is qualitatively identical. When the extremity is lowered the vasoconstric... response to increase in vascular transmural pressure is more pronounced in the leg. ...the vessels of the leg are subjected to higher transmural pressures the present findings ...in agreement with results reported by Folkow *et al.* (1958), Conway (1963), and Siverts... (1970). During elevation blood flow remains constant confirming that autoregulation ...blood flow is present in human subcutaneous adipose tissue (Henriksen, Nielsen, and ...ske 1973).

...I-Antipyrine washout is influenced by four components, *viz.* washout from cutaneous ...tissue, washout from subcutaneous adipose tissue, transport of tracer from cutaneous to ...cutaneous tissue, and trauma of injection. k_{max} was estimated from the initial slope of the ...I-Antipyrine washout curve. The interceptions at time zero of the monoexponential tail ...of the curves obtained in hand and foot accounted for about 10 and 20 per cent of the ...total height of the washout curve, respectively. This indicates that the initial washout rate

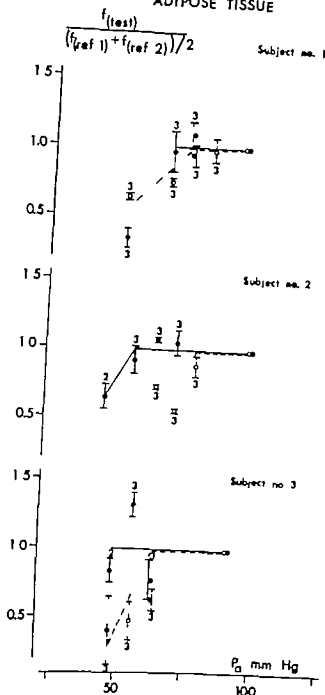


Fig. 2. Relative changes of blood flow ($f_{(te}$… $((f_{ref} + f_{ref\ 2})/2)$ as a function of m… arterial pressure ($\bar{P}$). Open circles … the broken line refer to experiments … the distal part of forearm, filled circ… and the full line to experiments on th… distal part of crus. The horizontal ba… signify 1 S.E.

By comparing k_{max} obtained from hand and foot after 20, 30 and 35 min of ischemia, the difference in responses was significant at the 95 per cent level ($p < 0.02$ by randomization test for paired samples). During 35 min of ischemia skin temperature of the areas under study increased about 1.5°C. Maximum washout rate constant times duration of hyperemia (k_{max} t) can be applied in calculating cumulative blood flow ("repayment") (Fig. 4). There was significant correlation between k_{max} t and duration of ischemia. The Spearman rank

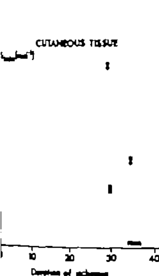


Fig. 3



Fig. 4

3. The maximum washout rate constant (k_{max}) of [131]I Antipyrine washout from cutaneous tissue as function of duration of ischemia. Open circles denote hand, filled circles foot. Each square represents the mean k_{max} values between hand and foot in one experiment.

4. The product of maximum washout rate constant times duration of hyperemia (k_{max} t) as function of duration of ischemia. Open circles denote hand, filled circles foot. The Spearman rank correlation coefficient, r_s 0.99 and 0.91 for hand and foot ($p < 0.01$ one-sided significance level, for both regions).

relation coefficient, r_s, was 0.99 (p 0.01) for hand and 0.91 ($p < 0.01$) for foot (one sided significance level). k_{max} t of hand and foot did not differ (randomization test for paired observations, p 0.9).

Discussion

The influence of orthostatic pressure changes on blood flow in subcutaneous adipose tissue of forearm and leg is qualitatively identical. When the extremity is lowered the vasoconstrictor response to increase in vascular transmural pressure is more pronounced in the leg. As the vessels of the leg are subjected to higher transmural pressures the present findings are in agreement with results reported by Folkow *et al.* (1958), Conway (1963), and Sivertsen (1970). During elevation blood *flow remains constant confirming that autoregulation* of blood flow is present in human subcutaneous adipose tissue (Henriksen, Nielsen, and ... 1973).

[131]I-Antipyrine washout is influenced by four components, *viz.* washout from cutaneous tissue, washout from subcutaneous adipose tissue, transport of tracer from cutaneous to subcutaneous tissue, and trauma of injection. k_{max} was estimated from the initial slope of the [131]I-Antipyrine washout curve. The interceptions at time zero of the monoexponential tail part of the curves obtained in hand and foot accounted for about 10 and 20 per cent of the total height of the washout curve, respectively. This indicates that the initial washout rate

from cutaneous tissue is underestimated by less than 10 and 20 per cent in hand and f respectively The difference in maximum hyperemic response in hand and foot migh due to different contribution of the washout component for subcutaneous adipose tis However the difference in interception values of about 10 per cent cannot explain difference in maximum hyperemic response of some 60 per cent. Cutaneous blood flow measured just distal to the cuffs to minimize steal of blood flow from skin to muscle interfere with the results. It is assumed that the vessels were maximally dilated in both reg as prolonged duration of ischemia did not increase the maximum washout rate cons any further As hand and foot were placed at the same level in relation to the heart, art perfusion pressures in the two areas are considered to be almost identical.

k_{max} t is influenced by the injection trauma. This seems likely from the results preser in Fig. 3 where the washout rate constant obtained in leg after 10 min of ischemia is alm similar to that obtained after 35 min of ischemia. The injection trauma is presumably of same order of magnitude in all experiments because the traumatically liberated metabol are not removed until the cuffs are deflated. Therefore, the calculated k_{max} t gives an o estimation of repayment" No difference in "repayment" was found in the two regi examined ($p > 0.9$).

As cutaneous tissue to blood partition coefficient of I Antipyrine is about 1 ml/g, bl flow in cutaneous tissue during maximum hyperemia is about 40 ml/100 g min in hand a about 25 ml/100 g min in foot. In subcutaneous adipose tissue of the crus maximum bl flow was 15 ml/100 g min after 30 min of ischemia (Nielsen and Sejrsen 1972). In musc tissue Lindbjerg (1966) reported values of 60 ml/100 g min. The findings indicate t vascular resistance during maximum dilatation is more pronounced in vessels subjected orthostatic hypertension. Findings by Folkow *et al* (1958, 1973) by Conway (1963), and Sivertsson (1970) are in accordance with this.

Folkow *et al* (1958 1973) ascribe difference in reaction to adaptive structural chan in the wall of vessels subjected to increased transmural pressure, which creates a vasc "hyperreactivity" Results obtained after histometrical examination of the vessels are accordance with this theory (Furuyama 1961). The findings in the present study indic that functional changes of vessels subjected to increased blood pressure are present in norm subjects. Therefore, the structural changes of vessel walls seem to be a normal physiologi adaptation in vessels subjected to increased transmural pressure.

The authors express their gratefulness to Dr Per Sejrsen for dvice, discussions, nd criticism of the ma script.

References

CONWAY J A vascular abnormality in hypertension. *Circulation* 1963 27 520–529

FOLKOW B., G. GRIMBY and O THULESIUS, Adaptive structural changes of the ascular alls in hyp tension and their relation to the control of the peripheral resistance. *Acta physiol scand.* 1958. 44, 2 272.

FOLKOW B., M HALLBÄCK, Y LUNDGREN, R. SIVERTSSON, and L. WEISS, Importance of adaptive chan in vascular design for establishment of hypertension, tudied n man and in spontaneously hypertens rats. *Circulat Res.* 1973. 32 33. Suppl. I. 1.2 1 13.

[illegible], M., Histometrical investigations of arteries in reference to arterial hypertension. *Tohoku J. exp. Med.* 1962, 76, 388–414.

[illegible], O., S. L. Nielsen and W. P. Paaske, Autoregulation of blood flow in human adipose tissue. *Acta physiol. scand.* 1973, 89, 531–537.

[illegible], O., S. L. Nielsen, W. P. Paaske and P. Sejrsen, Autoregulation of blood flow in human [illegible] tissue. *Acta physiol. scand.* 1973, 89, 538–543.

[illegible], S. S., Theory and applications of the exchange of inert gas at the lungs and tissues. *Pharmacol. Rev.* 1951, 3, 1–41.

[illegible], I. F., Leg muscle blood-flow measured with [133]Xenon after ischaemia periods and after muscular work performed during ischaemia. *Clin. Sci.* 1966, 30, 399–408.

[illegible], S. L., Adipose tissue blood flow determined by the washout of locally injected [133]Xenon. *Scand. J. clin. Lab. Invest.* 1972, 29, 31–36.

[illegible], S. L. and P. Sejrsen, Reactive hyperemia in subcutaneous adipose tissue in man. *Acta physiol. scand.* 1972, 85, 71–77.

[illegible], P., *Measurement of cutaneous blood flow by freely diffusible radioactive isotopes.* Thesis. Copenhagen 1971. *Danish Medical Bulletin.* 1971, Suppl. 18.

[illegible], R., The haemodynamic importance of structural vascular changes in essential hypertension. *Acta physiol. scand.* 1970, Suppl. 343.

Acta physiol. scand. 1975. 95. 470–476
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Pulsatile Secretion of ACTH, GH, LH and TSH in Man

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Abstract

LEPPÄLUOTO J U LAISI H LYBECK, J PARTANEN, T RANTA and P VIRKKUNEN. P secretion of ACTH GH LH and TSH in man. Acta physiol. scand. 1975 95 47

Blood samples were taken every 4 min for 80 min f om 5 healthy subjects. Plasma ACTH, GH, l TSH were estimated by radioimmunoassays. The mean peak intervals were 11 12, 13 and 14 respect vely and mean peak amplitudes were 25 pg, 1 1 ng, 11 ng and 5.3 μU Mean durations of as and descending limbs of the plasma patterns of these hormones were also measured. The timing different hormone peaks appeared to be independent

When several blood samples are drawn at short intervals and estimated for hormone centrations, the blood hormone levels fluctuate continuously This phenomenon was observed in the secretion of ACTH by Berson and Yalow (1968). Since then experim evidence has accumulated that other hormones, e.g cortisol (Hellman et al 1970), (Takahashi et al 1968) FSH and LH (Naftolin et al 1972) LRH (Jeffcoate et al 1974) TSH (Vanhaelst et al 1972) also fluctuate instead of remaining at a constant level in plasma. The intermittent increases of the serum hormone levels are called frequent spi bursts, peaks or secretion episodes, and are believed to result from simultaneous releas discrete hormone packets from secreting cells into the circulation. Electron micros studies on the adenohypophysis support this view because the hormones have been fo to be packed in electron-dense granules, which disappear during the secretory phase (Szent gothal et al 1968).

The secretion bursts of adenohypophyseal hormones are evidently initiated by the libe tion of releasing hormone, and triggered by the release of neurotransmitters in the hy thalamus. These fluctuations of blood hormone levels have interesting physiological clinical corollaries the secretion bursts of gonadotrophins develop in adolescence and amplified after the menopause (Yen et al 1973). In certain amenorrheas the fluctuation subdued, but in gonadal dysgenesis it is enhanced (Yen et al 1973). Similarly clomiphe a drug inducing ovulation, increases the frequency and amplitude of LH secretion bur (Boyar et al 1973). In previous studies, where samples were taken at 20–60 min interval only 6–14 secretion periods per day were found (Boyar et al 1973 Hellman et al. 1970

xperimental conditions, however rhythms with a frequency of less than 20 min be detected. When samples were taken more frequently (every 1–3 min) from bjects, a secretion burst of plasma cortisol and GH was observed every 5–10 min l. 1973).

sent results show that the plasma concentrations of ACTH, LH and TSH as of time imply very short intervals between secretion episodes caused by inter *ions of substantial amounts of these hormones into the blood.*

Material and methods

healthy subjects aged 24–32 years (4 males and 1 female) participated in the experiments. They ers of the laboratory staff, and four of them (the males) had previously taken part in similar s (Lam et al. 1973).

ss. The experiments were started at 10–11 a.m. A small area above the antecubital vein was esthetized (Lidocain® 2 %, Orion, Finland, 0.3 ml s.c.) and some minutes later cannula Viggo AB, Sweden) was inserted into the vein. The subject then sat in chair in the laboratory l min for 80 min 10-ml blood sample was rapidly (maximally in 1 min) withdrawn into plastic containing 10 U. of heparin per ml. No one felt pain during the venipuncture, but subject No. 5 l of vertigo at the beginning of the experiment.

mmunoassay. The plasma samples were stored at −20 C for 1–4 months before the estimations. CTH and GH were assayed with commercial kits (Amersham, U.K. and Kabi, Sweden) and LH with reagents obtained from NIAMDD, Maryland, Bethesda, USA. The sample volume for the A was 1 ml, for GH 0.1 ml, and for LH and TSH 0.2 ml. Every sample was assayed in dupl cat H the division into two was done after the extraction procedure). Each hormone was estimated licate except ACTH, which was assayed in four replicates. The immunocomplex formed by the and antiserum was separated on charcoal in the ACTH and GH radioimmunoassays and by antibody method in the LH and TSH radioimmunoassays. The standard curves in the ACTH radioimmunoassays were set up in hormone-free plasma, the others in buffer. The statistical tics of the RIAs are presented in the following table (Table I). A significant change in hormone was defined as change of two or more ± S.D. The area of tw ± S.D. covers 99.5 of the values ry.

Results

ical temporal patterns of short-term secretion of ACTH GH, LH and TSH are d in Fig. 1 and 2 (subjects 1 and 2). ACTH secretion exhibits two high spikes and ller peaks.

asma GH level in subject 5 (Fig. 2) was extremely high at the beginning of the ent but then declined, fluctuating to the normal level. Although this subject com of vertigo when the cannula was inserted, her ACTH secretion was unchanged he first 16 min.

a LH and TSH displayed from 2 to 4 significant secretion peaks in subjects 1 and 5 he 80-min experiment (Fig. 1 and 2).

detailed values for the secretion events in 5 subjects are presented in Table II.

s study each hormone is secreted in 15–19 significant bursts with a mean interval of in. The mean peak amplitude in ACTH secretion was 25 pg, in GH secretion 1.1 ng, secretion 11 ng and in TSH 5.3 µU The respective percentages of the overall mean s alues are 60, 25, 30 and 90 % (Table I). The secretion of GH and especially ACTH

TABLE I.

Hormone	Standard	Intra-assay coefficient of variation	Linear range of the inhibition curve	Normal value	2 S D of t estimate
ACTH	α 1 4 ACTH	10.5 %	10–400 pg/ml R = 0.94	10–75 pg/ml	10 pg/ml
GH	a Kabi standard	3.3 %	1.2 20 ng/ml R = 0.98	0.2–8 ng/ml	0.5 ng ml
LH	LER 907	10 3 %	15–1 000 ng/ml R = 0.99	20–56 ng/ml	6 ng/ml
TSH	MRC 63/68	15 5 %	0.78–50 μU/ml R = 0.98	2 10 μU/ml	4 μU/ml

[1] Four separate ACTH radioimmunoassays were made the interassay coefficient of variation was 1 4
[2] R = regression coefficient biding percentage (of total) as dependent variable and $\log_{10}$ hormone c centration as ndependent variable. R was always h ghly significant.

could be divided into low and high peaks, the latter appearing and disappearing rapi (Fig. 1 and 2 and Table II)

The frequency of coinciding peaks was also recorded, but the timing of the differe hormone peaks appeared to be independent For example, GH secretion coincided with L peaks 6 times, with TSH peaks 5 times, and with ACTH 4 times, and appeared indepe dently 4 times. When correlations were calculated between every possible pair of plasm hormone levels measured from the same sample for each subject, the only significant correl ions were between the LH and TSH levels in two subjects (No 2 and 3).

The distribution of the hormone concentrations was skewed to the right in every ca Fig. 3), skewness being greatest in the ACTH and GH concentrations. These high ACTI nd GH concentrations correspond to the high peaks seen in their secretion.

We regard the "normal" range of values for ACTH as 10–75 pg/ml and during rapi maximally 1 min) blood sampling there is about a 10 % chance of hitting on a high secretio eak, in which the values reach 150–250 pg/ml In GH secretion our estimates for normal" alues range from 0.2 to 8 ng/ml and there is a similar 10 % chance of obtaining GH value to 40 ng/ml The two SD ranges for LH and TSH were 20–56 ng/ml and 2–10 μU/ml, espectively

Discussion

he major findings of the present study can be summarized as follows 1) Significant peaks the plasma concentrations of ACTH, GH LH and FSH were found every 11 14 min neans) 2) the mean peak amplitudes varied from 20 to 160 % (maximum even 1 000 %) of e pre-peak levels. 3) The duration of the peaks varied from 8 to 14 min. 4) The secretion eaks of the four hormones occurred independently

The rapid increases and decreases in plasma hormone concentrations imply that each type adenohypophyseal cell releases discrete packets of its specific hormone intermittently into e blood. Dye dilution studies have shown that a dye takes about 10 s to appear in the blood d that mixing is complete within 6 min (Williams and Deegan 1971). There is no reason to

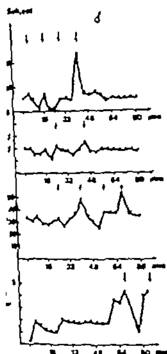


Fig. 1

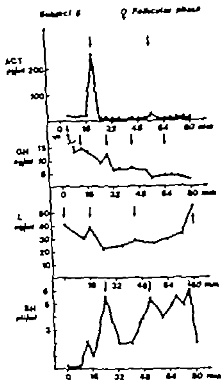


Fig. 2

...and 2. Typical temporal patterns of short-term secretion of ACTH, GH, LH and TSH.

...that the duration of a hormone burst would differ in any respect during the first few ...tes. *In fact, the high secretion peaks of ACTH found in this study conform well to* ...ults obtained by the dye dilution technique. The longer duration of certain secretion ...s can be due *to our relatively long sampling interval, which would not allow us to detect* ...e peaks and troughs in the hormone dilution curve. There is indirect evidence that the ...tion bursts of the adenohypophyseal hormones are initiated by the releasing hormones ...triggered by the hypothalamic neurotransmitters. For example, the plasma LRH con-...ntration in sheep has been demonstrated to indicate brief secretion episodes (Jeffcoate ...t. 1974). Although at the moment there is no direct evidence that the secretion of hypo-...amic neurotransmitters, releasing hormones and adenohypophyseal hormones are in ...causal relationship suggested, this appears highly probable. If there were no "external" ...chronizers, the trophic cells would secrete at random and produce a constant plasma level ...hormone.

ACTH, GH, LH and TSH evidently have different releasing and inhibiting factors and are ...ject to different hypothalamic neurotransmitter systems, and hence it was expected that ...secretion peaks of these hormones in the same plasma samples would occur indepen-...ntly. The correlation between the plasma LH and TSH levels found here in two subjects ...probably due to contamination of LH antiserum with TSH antibodies, as suggested before

TABLE I

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Four separate ACTH radioimmunoassays were made, the interassay coefficient of variation was 1–4 %. R = regression coefficient, binding percentage (of total) as dependent variable and $\log_{10}$ hormone concentration as independent variable. R was always highly significant.

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The frequency of coinciding peaks was also recorded, but the timing of the different hormone peaks appeared to be independent. For example, GH secretion coincided with LH peaks 6 times, with TSH peaks 5 times, and with ACTH 4 times, and appeared independently 4 times. When correlations were calculated between every possible pair of plasma hormone levels measured from the same sample for each subject, the only significant correlations were between the LH and TSH levels in two subjects (No. 2 and 3).

The distribution of the hormone concentrations was skewed to the right in every case (Fig. 3), skewness being greatest in the ACTH and GH concentrations. These high ACTH and GH concentrations correspond to the high peaks seen in their secretion.

We regard the "normal" range of values for ACTH as 10–75 pg/ml, and during rapid (maximally 1 min) blood sampling there is about a 10 % chance of hitting on a high secretion peak, in which the values reach 150–250 pg/ml. In GH secretion our estimates for "normal" values range from 0.2 to 8 ng/ml and there is a similar 10 % chance of obtaining GH values up to 40 ng/ml. The two SD ranges for LH and TSH were 20–56 ng/ml and 10 μU/ml, respectively.

Discussion

The major findings of the present study can be summarized as follows. 1) Significant peaks in the plasma concentrations of ACTH, GH, LH and FSH were found every 11–14 min (means). 2) the mean peak amplitudes varied from 20 to 160 % (maximum even 1 000 %) of the pre peak levels. 3) The duration of the peaks varied from 8 to 14 min. 4) The secretion peaks of the four hormones occurred independently.

The rapid increases and decreases in plasma hormone concentrations imply that each type of adenohypophyseal cell releases discrete packets of its specific hormone intermittently into the blood. Dye dilution studies have shown that a dye takes about 10 s to appear in the blood and that mixing is complete within 6 min (Williams and Deegan 1971). There is no reason to

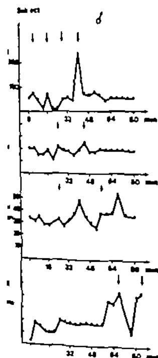


Fig. 1

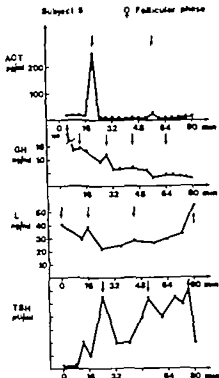


Fig. 2

...and 2. Two typical temporal patterns of short-term secretion of ACTH, GH, LH and TSH.

...ose that the dilution of a hormone burst would differ in any respect during the first few ...tes. In fact, the high secretion peaks of ACTH found in this study conform well to ...sults obtained by the dye dilution technique. The longer duration of certain secretion ...s can be due to our relatively long sampling interval, which would not allow us to detect ...le peaks and troughs in the hormone dilution curve. There is indirect evidence that the ...tion bursts of the adenohypophyseal hormones are initiated by the releasing hormones ...riggered by the hypothalamic neurotransmitters. For example, the plasma LRH con-...tration in sheep has been demonstrated to indicate brief secretion episodes (Jeffcoate ...l 1974). Although at the moment there is no direct evidence that the secretion of hypo-...lamic neurotransmitters, releasing hormones and adenohypophyseal hormones are in ...osed relationship suggested, this appears highly probable. If there were no "external" ...dulators, the trophic cells would secrete at random and produce a constant plasma level ...hormone.

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TABLE II

Hormone peak range	Number of significant peaks	Mean peak interval	Mean peak amplitude	Mean duration of ascending/descending limb
ACTH				
10–100 pg	12	11 min	25 pg	6 min/6 min
over 100 pg	7			4 min/4 min
GH				
0.5– ng	11	12 min	1 1 ng	5 min/11 min
over 2 ng	8			
LH				
5–20 g	15	13 min	11 ng	6 min/11 min
TSH				
4–12 μU	18	14 min	5.3 μU	8 min/10 min

Total observation time 400 min.

(Aubert and Lemarchand-Beraud 1971) Our calculations on the summated duratior ascending limbs of the secretion peaks indicate that the adenohypophyseal cell secre during 1/5–1/3 of the time, divided into several episodes per hour After complete of an adenohypophyseal hormone in the blood circulation its metabolic half-life h

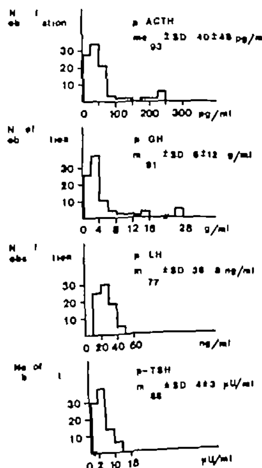


Fig. 3 The distribution of hormone concen The total observation time as 400 min.

shown to vary from 20 to 60 min (Daughaday 1974). The rapid dilution and slow elimination mone result in relative rarity of prominent hormone peaks, i.e. positive skewness of cy distribution of hormone assays. Skewness was greatest in the case of peptide es such as ACTH and GH, but did not noticeably disturb the calculation of the l values for TSH and LH. The ACTH and GH distribution curves were twopeaked, al descents from the high levels representing the hormone-dilution effect. As normal for plasma ACTH and GH we estimate 10–75 pg/ml and 0.2–8 ng/ml, respectively into account that the probability of obtaining a single elevated value by chance is 10%. Such a misleading measurement can be avoided by taking several blood samples sample extremely slowly ... in 10 min.

estimation of normal values for GH in this study presented problems. Subject No. 5 tly elevated plasma GH levels at the beginning of the experiment, evidently due to ss caused by insertion of the cannula. However her ACTH levels were not elevated time, showing that ACTH and GH are secreted independently. Subject No. 4 and the 3 subjects had previously participated in a similar experiment and on both occasions d elevated plasma GH levels. In this and the previous study the other subjects had GH levels lower than 4 ng/ml in altogether 115 plasma samples. The height and weight GH-hypersecretor are normal, as well as his blood sugar level, and he has no clinical of hypersecretion of GH. Hence our normal values for plasma GH are somewhat but we have no reason to exclude these subjects from our series.

showed previously that in the resting state, between wakefulness and dozing, the ges in central nervous activity as measured by EEG vigilance, were correlated with bursts of GH secretion, but not with burst of cortisol secretion (Lahi *et al.* 1973). s study the subjects were awake throughout the experiment and probably at the same EEG vigilance level, but even so had short secretion bursts in their plasma hormone ntrations. It may be that the central nervous system has a permissive effect on the lamo-pituitary unit.

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References

...T, M. L. and T. LEMARCHAND-BERAUD, J. *Radioimmunoassay methods.* Eds. K. Kirkham and Hunter Edinburgh: Churchill Livingstone. 1971 232–.39

...N, S. A. and R. S. YALOW Radioimmunoassay of ACTH in plasma. *J. clin. Invest.* 1968. 47 2725–...

...S, R. M., M. PERLOW, S. KAPEN, G. LEFKOWITZ, E. WEITZMAN and L. HELLMAN, The effect of clomiphene citrate on the 24-hr LH secretory pattern in normal men. *J. clin. Endocr.* 1973. 36, 561–567

...DAY, W. H., In *Textbook of endocrinology.* Ed. R. H. Williams. Philadelphia. W. B. Saunders Co. ...4. 31–79

...MAN, L., F. NAKADA, J. CURTI, E. D. WEITZMAN, J. KREAM, H. ROFFWARG, S. ELLMAN, D. K. FUKUSHIMA and T. F. GALLAGHER, Cortisol is secreted episodically by normal man. *J. clin. Endocr.* 1970. 30, ...–422.

...ATT, S. L., H. M. FRASER, D. T. HOLLAND and A. GUNN, Radioimmunoassay of luteinizing hormone-releasing hormone (LH RH) in serum from man, sheep and rat. *Acta endocr.* (Kbh.) 1974. 75 625–635.

..., U., S. LAHTI, J. LEPPÄLUOTO, J. LUMIO, J. PAR... and P. VIRKKUNEN, Growth hormone and ...ol secretion in ma... *Acta physiol. scand.* 1973. 89 192–197

NAFTOLIN, F., S. S. C. YEN and C. C. TSAI, Rapid cycling of plasma gonadotrophins in normal men demonstrated by frequent sampling. *Nature* (Lond.) 1972. *236* 92 93.

SZENTAGOTHAI, J B FLERKO, B. MESS and B. HALASZ, In *Hypothalamic control of the anterior pituitary* Budapest Akademiai Kiado. 1968. 69

TAKAHASHI, Y., D M. KIPNIS and W H. DAUGHADAY Growth hormone secretion during sleep. *J clin. Invest* 1968. *47* 2079–2090.

VANHAELST L. E. VAN CAUTER, J P DEGAUTE and J GOLSTEIN, Circadian variations of serum thyrotropin levels in man. *J clin. Endocr* 1972. *35* 479–482.

WILLIAMS, M J and T DEEGAN, ⁹⁹ᵐTc labelled serum albumin in cardiac output and blood volume studies. *Thorax* 1971 *26* 460–465

YEN, S. S. C., R. REBAR, G VANDENBERG and H JUDD Hypothalamic amenorrhea and hypogonadotropinism. Responses to synthetic LRF *J clin. Endocr* 1973 *36*. 811–816.

Acta physiol. scand. 1975. 95. 477—481

From the Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden

Effect of Bulbar Acidification on Basal Secretion of Acid and Gastrin in Dog

By

GÖRAN NILSSON

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Abstract

...on, G. *Effect of bulbar acidification on basal secretion of acid and gastrin in dog.* Acta physiol. scand. 1975. 95. 477–481

...was provided with mucosal septal pouches of the stomach and of the duodenal bulb. In some dogs ...large gastric cannula was inserted into the most dependent portion of the stomach. In dogs which ...found to secrete acid spontaneously during control period at the start of each experiment, the bul... ...pouches were perfused with 0.1 M HCl for 5–120 min. Bulbar acidification rapidly and profoundly ...ed the basal acid output. In dogs which did not secrete acid spontaneously during the control period ...r pouches were perfused with 0.1 M HCl for 1 h. Bulbar acidification did not significantly influence ...basal gastrin concentration and no acid was secreted from the Pavlov pouches following such acidi... ...on. The present results support the hypothesis that reduction of the intrabulbar pH may contribute ...r reduction of acid secretion during interdigestive periods. The physiological significance of mecha... ...s in the upper intestine which induce acid secretion following reduction of the intraluminal pH is ...ssed.

...ction of the intraluminal pH in the duodenal bulb activates a mechanism which is ...effective in *inhibiting gastric acid secretion induced by various stimuli* (Andersson and ...ls 1961, Nilsson 1969 a). The inhibitory mechanism is activated at pHs which normally ...t in the proximal portion of the dog duodenum during postprandial and interdigestive ...ods. The present study was undertaken to ascertain whether acid perfusion of isolated ...ar pouches inhibits spontaneous acid secretion from Pavlov pouches in dog. There is ...erimental evidence that acidification of the mucosa of the upper intestine induces acid ...tion (Ivy and McIlvain 1923, Sircus 1953, Code and Watkinson 1955, Sircus 1958). It ...therefore decided *to investigate* whether or not acid perfusion of bulbar pouches in... ...ts acid secretion in Pavlov pouch dogs. In addition, the possible influence of bulbar ...ification on plasma gastrin levels in dogs was studied.

Methods

...ical procedures

...s weighing 12–18 kg were prepared with mucosal septal pouches (Pavlov type) of the stomach and of ...proximal portion of the duodenum, corresponding to the duodenal bulb (Andersson and Uvnäs 1961).

ıFTOLIN, F S. S. C. YEN and C. C. TSAI, Rapid cycling of plasma gonadotrophins in normal men demonstrated by frequent sampling. *Nature* (Lond) 1972. 236, 92 93
ENTAOOTHAL, J B. FLERKO, B. MESS and B. HALASZ, In *Hypothalamic control of the anterior pituitary* Budapest. Akademiai Kiado 1968. 69
KAHASHI, Y., D M. KIPNIS and W H DAUGHADAY Growth hormone secretion during sleep. *J clin Invest* 1968. 47 2079–2090.
ıNHAELST L., E. VAN CAUTER, J P DEGAUTE and J GOLSTEIN Circadian variations of serum thyrotropin levels in man. *J clin. Endocr* 1972. 35 479–482.
ILLIAMS, M J and T DILGAN ^{99m}Tc labelled serum albumin in cardiac output and blood volume studies. *Thorax* 1971 26 460–465.
N, S. S. C., R REBAR, G VANDENBERG and H JUDD, Hypothalamic amenorrhea and hypogonadotropinism. Responses to synthetic LRF *J clin. Endocr* 1973. 36 811–816.

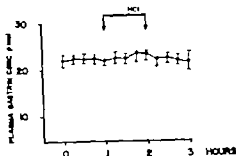


Fig. Plasma gastrin concentration before, during and after perfusion of isolated bulbar pouches with 0.1 M HCl. Each symbol represent the mean of 9 expts. in 3 dogs. Vertical bars represent the range.

... "Experimental series". Following perfusion with acid, the pH of the effluent perfusate varied between 1.1 and 1.3. Blood samples were collected from a leg vein at 15 min intervals. 4–6 blood samples were collected for the determination of basal plasma levels of gastrin. Four samples were taken during the perfusion with acid and another four to six samples after completion of the acid perfusion. The blood was drawn into syringes that had been moistened with heparin. Approximately 100 U of heparin (generously supplied by Vitrum AB) was added per 4 ml of blood. The blood was then centrifuged immediately and the plasma was removed, frozen and kept at −20° until its gastrin content was determined by radioimmunoassay. The method used for the radioimmunoassay of gastrin has been described elsewhere (Nilsson 1975 a).

Experimental series

Two series of experiments were undertaken.

Series A was performed on Pavlov pouch dogs whose basal acid output during the control hour was at least 15% of the maximal acid output to histamine. In these dogs the bulbar pouches were perfused with 0.1 M HCl for 5–120 min at a rate of 60 ml per hour.

Series B was undertaken to determine if acid perfusion of bulbar pouches with 0.1 M HCl influences the basal plasma concentration of gastrin or reduces acid secretion. The dogs used in this series did not secrete acid spontaneously from their Pavlov pouches during the control hour at the start of the experiment. To avoid any gastric juice that may have been present in the main stomach entering the pyloric region and the intestine the gastric cannula was kept open during the entire experiment. After a control period of 1 h, the bulb was perfused with 0.1 M HCl for 1 h. Acid secretion and plasma levels of gastrin were followed during the period of bulbar acidification and after stopping the perfusion for another 1 to 2 h.

Results

Series A

Acid perfusion of bulbar pouches profoundly inhibited the fasting acid secretion from the Pavlov pouches. A short period of bulbar acidification was sufficient to evoke inhibition and maintaining a low pH in the bulbar pouches produced a sustained inhibition of acid secretion. These results are illustrated in Fig. 1

Series B

Basal plasma gastrin concentrations were not significantly changed following acidification of the bulbar pouches in 9 experiments in 3 dogs (Fig. 2).

No acid was secreted during the hour preceding the bulbar acidification in these dogs, and the acid perfusion of the bulbar pouches in these or 2 other dogs (in all 15 expts) did not give rise to any secretion of acid during either the perfusion or post-perfusion hours. These negative results are not illustrated.

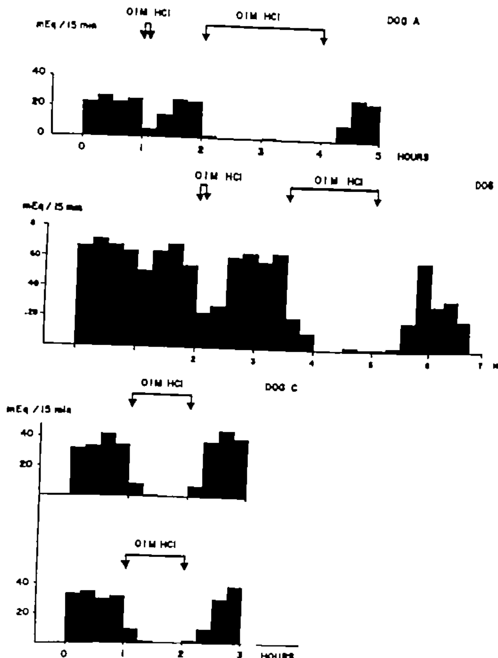


Fig. 1 Basal acid output from Pavlov pouch dogs bef re, during and after perfusion of bulbar po with 0.1 M HCl for 5 60 and 120 min.

In addition, three of the dogs were provided w th a gastric cannula inserted into the most dependent tion of the stomach. Between each operation nd before the experimental studies were started the dogs allowed 3–4 weeks for recovery

Experimental procedures

Experiments were started in the morning after the dogs had been fasted for 18–20 h. Basal acid ou was recorded for one hour the secretory responses being collected n 15 min samples. The olume measured and the acidity was determined by titratio w th 0.01 M NaOH using phenolphthalein as dicator Perfusion of the bulbar pouches with solutions of 0.1 M HCl was performed as described be

r pH, it is not localized to the bulbar portion of the duodenum. In the postbulbar denum of the dog the intraluminar pH does not reach low levels either during fasting ostprandial periods (Brooks and Grossman 1970). It is therefore questionable if reduc of the intraluminar pH of the postbulbar portion of the intestine under physiological ditions contributes to the stimulation of gastric acid secretion at all.

n summary the present results demonstrate that reduction of the intrabulbar pH pro- ndly inhibits spontaneous gastric acid secretion from Pavlov pouches. The results sup- t the concept that acidification of the bulbar mucosa plays a role in inhibiting gastric d secretion occurring during interdigestive periods. Acid perfusion of bulbar pouches ler basal conditions does not induce secretion of acid in Pa lov pouch dogs.

s study was supported by research grants from Statens Medicinska Forskningsråd (project No. 3521) from Karolinska Institutet. The author is grateful to Miss Gunilla Thulin, Miss Elisabeth Tarén and s Iust Bichtorvski for technical and secretarial assistance.

References

ANDERSSON, S. and G. NILSSON, pH-dependence of the mechanism in the duodenal bulb inhibiting gastric acid response to exogenous gastrin. Acta physiol. scand. 1969. 76. 182 190.
ANDERSSON, S. and L. SJÖDIN, Inhibition of gastric secretion by acidification of the duodenal bulb before and after transection of the pylorus. Scand. J. Gastroent. 1972. 7 151 156.
ANDERSSON, S. and B. UVNÄS, Inhibition of gastric secretion in Pavlov pouches by instillation of hydrochloric acid into the duodenal bulb. Gastroenterology 1961 41 486–490.
ANDERSSON, S., G. NILSSON and B. UVNÄS, Effect of acid in proximal and distal duodenal pouches on gastric secretory responses to gastrin and histamine. Acta physiol. scand. 1967 71 368–378.
BROOKS, A. M. and M. I. GROSSMAN, Postprandial pH and neutralizing capacity of the proximal duodenum in dogs. Gastroenterology 1970. 59 85–89.
GREGORY, C. F. and G. WATERHOUSE, Importance of vagal innervation in the regulatory effect of acid in the duodenum on gastric secretion of acid. J. Physiol. (Lond.). 1955. 130. 233–252.
IVY, A. C. and G. B. MCILVAIN, The excitation of gastric secretion by application of substances to the duodenal and jejunal mucosa. Amer. J. Physiol. 1923 67 124–140
NILSSON, G. Studies of the mechanism by which acid in the duodenal bulb inhibits gastric acid secretion. Dissertation (Stockh.) 1969 a.
NILSSON, G., Effect of acid in the duodenal bulb on gastric secretory responses to insulin hypoglycemia. Acta physiol. scand. 1969 b. 73 476–483.
NILSSON, G. Effect of acid in the duodenal bulb on gastric secretory responses to sham feeding. Acta Physiol. scand. 1969 c. 77 308–315
NILSSON, G. Effect of acidification of totally denervated pouches of the duodenal bulb on gastric acid secretion in dog. Acta physiol. scand. 1974. 92 569–571
NILSSON, G., Increased plasma gastrin levels in connection with inhibition of gastric acid responses to sham feeding following bulbar perfusion with acid in dogs. Scand. J. Gastroent. 1975 a. 10 273–278
NILSSON, G., Effect of acid in the duodenal bulb on acid and pepsin responses to test meal in dog. Scand. J. Gastroent. 1975 b. 10. 279–282
NILSSON, G. Effect of bulbar perfusion with acid, hypertonic solutions and acetylcholine on gastric acid secretion in dogs. Scand. J. Gastroent. 1975 c. 10. 283–287
NILSSON, G., Effect of bulbar acidification on gastric acid responses to urecholine in Pavlov pouch dogs. Acta physiol. scand. 1975 d. 95 258–262.
NILSSON, G. and S. RUNE, Failure of high PCO_2 in the duodenal bulb to inhibit gastric acid secretion. Acta physiol. scand. 1971 81 349–354.
NILSSON, G., J. SIMON, R. S. YALOW and S. A. BERSON, Plasma gastrin and gastric acid responses to sham feeding and feeding in dogs. Gastroenterology 1972. 63. 51 59
SIRCUS, W. The intestinal phase of gastric secretion. Quart. J. Exp. Physiol. 1953. 38. 91 99
SIRCUS, W. Studies on the mechanisms in the duodenum inhibiting gastric secretion. Quart. J. Exp. Physiol. 1958. 43. 114-133.

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Discussion

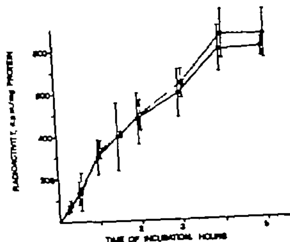
A series of studies have shown that the mechanism by which acid in the duodenum inh gastric acid secretion is mainly localized to the duodenal bulb (Andersson, Nilsson Uvnäs 1967) and that acid perfusion of bulbar pouches inhibits gastric acid secretio duced by a test meal (Andersson and Uvnäs 1961 Andersson and Sjödin 1972, Nil 1975 b) insulin hypoglycemia (Nilsson 1969 b Andersson and Sjödin 1972) sham fee (Nilsson 1969 c, Andersson and Sjödin 1972, Nilsson 1975 a) or exogenous gastrin (And son, Nilsson and Uvnäs 1967 Andersson and Nilsson 1969 Nilsson and Rune 1971 N son 1974 Nilsson 1975 c and d) Acid responses to histamine (Andersson, Nilsson and nås 1967) or to the stable choline ester urecholine (Nilsson 1975 d) are not inhibited by bulbar mechanism.

The present study shows that fasting acid secretion is profoundly reduced by acidificat of the bulbar mucosa in dogs. As in previous studies (Nilsson 1969 c) using sham feed as secretory stimulus, a short period of bulbar acidification was sufficient to produc significant reduction of the basal acid output. Similarly when acidification of the bul pouches was prolonged inhibition became more pronounced and was maintained as k as the pH in the bulbar pouch was kept low

In the present study the intrabulbar pH was reduced to 1 1–1.3 in all experiments. Ot studies show that the bulbar mechanism may be activated at considerably higher pl Thus, acid responses to low doses of gastrin (Andersson and Nilsson 1969) or to sham fe ing (Nilsson 1969 c) are reduced already at an intrabulbar pH of 3–4 Other experiments dogs (Brooks and Grossman 1970) show that the pH existing in the fasting duodenal bu is sufficiently low to activate a pH-sensitive inhibitory mechanism there. Therefore, t present results, together with the studies related above, strongly indicate that a reduction the intrabulbar pH may play a physiological role in inhibiting interdigestive acid secretic

The mechanism responsible for the acid secretion which occurs between meals is r known. Experiments in dogs show that atropinization does not significantly reduce the bas plasma gastrin concentration (Nilsson *et al* 1972) Such observations indicate that ba secretion of gastrin in dog is not under cholinergic control However those results do n exclude the possibility that under fasting conditions vagal stimulation may arise in the dc causing release of gastrin and cholinergic activation of the parietal cells which in turn r sults in the secretion of acid. Bulbar acidification liberates a humoral factor (Nilsson 197 which does not inhibit the release of gastrin but probably competes with gastrin at or clo to the parietal cells (Nilsson 1975 a). It seems probable that the inhibition of fasting ac secretion is accomplished in the same way

In the present study the effect of bulbar acidification on basal gastrin levels in dogs whi did not secrete acid spontaneously was also investigated. As found previously (Nilss 1975 a), perfusion of the bulbar pouches did not influence the basal plasma level of gastri like immunoreactivity Earlier studies (Ivy and McIlvain 1923 Sircus 1953 Code and Wa kinson 1955 Sircus 1958) indicate that perfusion of loops of the upper intestine with stro acid solutions induces secretion of acid from the stomach. Bulbar acidification did not evo acid secretion from the Pavlov pouches in the present study If there is a mechanism in th upper intestine of the dog that induces acid secretion following reduction of the intrab



. Comparison of amino acid incorporation into protein between slices by machine and hand. Slices of 80 mg in Hanks medium were incubated with 17 μM of ^{14}C-L-leucine (specific activity 34 mCi/mmol) for the indicated times cut by machine, by hand O---O The results are mean ± S.E. of 3 independent experiments, each run in duplicate.

further processing and identification. This was achieved by incubating slices under optimal conditions and on a large scale followed by saltfractionation and electrophoretic separation of the proteins.

A preliminary report of this work has been published (Wrodski and von der Decken 1973).

Materials and Methods

Chemicals. The chemicals used were of per analysis grade wherever possible. DL-leucine-1-^{14}C specific activity 34 mCi/mmol, L-valine-2,3-^{3}H specific activity 1.5 Ci/mmol and ^{3}H-amino specific activity 300 mCi/ and was obtained from the Radiochemical Centre, Amersham, Bucks, U.K. L-leucine-^{14}C (U) specific activity 250 mCi/mmol, L-leucine-4,5-^{3}H specific activity 5 Ci/mmol, L-amino acids-^{3}H (G) specific activity mCi/ml from NEN Chemicals GmbH, Frankfurt am Main, Germany. Soluene-350 from Packard Instrument Company, Downers Grove, Ill., U.S.A., Aquacide II from Calbiochem AG, Lucerne, Switzerland, Diaflo membrane PM 10 from Amicon Corp. Lexington, Mass., U.S.A., sterilized Hanks medium from Statens Bakteriologiska Laboratorium, Stockholm, Sweden, glass fiber discs Type A from Gelman Instrument Co., Ann Arbor, Michigan, U.S.A.

Animals. Sprague Dawley male or female rats of 90–130 g or mice of the N.M.R.I. strain of 20 g b.w. were used.

Tissue slices. The animals were decapitated. The brain was excised, placed in ice-cold medium and sliced. A comparison was made between chopping mechanically using a machine similar to that described by McIlwain and Buddle (1953), and slicing by hand. As determined with a micrometer the approximate thickness was 0.50–0.60 and 0.70–0.80 mm for slices cut by machine and hand respectively. The incorporation of ^{14}C-leucine into protein was similar for the two preparations (Fig. 1). In the following experiments the mechanical chopper was used.

(a) *Small scale incubation.* This was in Hanks or Krebs Ringer medium the mM composition of which is given in Table I (Hanks and Wallace 1949, Cohen 1951). 80 mg slices in 2 ml were incubated in 25 ml flasks at 35 with H_2O-saturated gas phase of O_2 CO_2 (95 5, v/v). A mixture of amino acids (Paul 1970) one of which was ^{14}C-labelled was added as indicated in the Figures and Tables. After incubation for the times indicated the mixture was transferred to an ice-cold teflon-glass homogenizer and homogenized thoroughly. Samples of 100 μl in triplicate were pipetted on to filter paper discs, the proteins were processed (Mans and Novelli 1961) and the radioactivity was measured (von der Decken 1968) in an Intertechnique Liquid Scintillation Spectrometer Model SL 30, at 90 efficiency.

To determine the extracellular free ^{14}C-L-leucine after incubation, the slices were centrifuged at 1 200 g for 1 min and washed three times with ice-cold medium containing 2 000-fold excess of unlabelled leucine the first washing medium. The slices were then homogenized in 2 ml of medium and 100 μl samples were pipetted on to filter paper discs. These were extracted for determination of radioactive proteins. T 1 ml

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From the Wenner-Gren Institute for Experimental Biology Stockholm, Sweden

Synthesis of Brain-Specific Acidic Proteins in Rat and Mouse Cerebral Slices

By

ANDRZEJ WROŃSKI AND ALEXANDRA VON DER DECKEN

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Abstract

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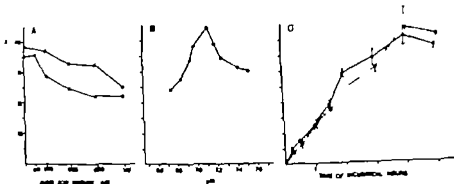
Optimal conditions for incorporation of radioactive amino acids into protein by slices of brain have be… established. Protein synthesis continued fo at least 4 h at 35 The highest incorporation of amino ac… into protein was obtained with 20–30 mg wet weight per ml incubated at pH 7 1 After incubation of 50 … of slices acidic proteins were separated by saltfractionation and electrophoresis on 14 % polyacrylamide g… The most acidic band was shown to contain S-100 protein. Identification was solubility of the protein … 100 % $(NH_4)_2SO_4$, electrophoretic mobility t pH 8.9 precipitation w th specific antibodies, and 2-dime… sional electrophoresis in polyacrylamide gel. The protein band was insoluble n 50 % methanol and contai… between 0 06 and 0.2 % of the radioactivity present in the total soluble proteins while other …ed separat… acidic proteins contained about 10 times the radioactiv ty of S-100 protein.

Key words. Brai slices, protein synthesis, S-100 protein, polyacrylamide gel electrophoresis

It has been reported (Hydén and Lange 1970) that some of the acidic brain proteins may b… specifically related to the process of learning. One of these proteins, S-100, has a molecul… weight of about 21 000 and contains a relatively high amount of glutamic and aspartic ac… (Moore and McGregor 1965). It is easily recognized by its solubility in ammonium sulfa… and characteristic electrophoretic migration at a pH higher than 8 (Moore 1965). The amou… present in the brain of young adult rats is about 0.2 % that of the total protein and the co… centration increases in hippocampal nerve cells after training (Hydén and Lange 1970). Th… apparent sensitivity of S-100 protein towards neurophysiological functions (Zuckerman… *et al.* 1970, DeRobertis 1967) would suggest that the protein is also affected by changes i… environmental conditions. These would influence primarily the synthetic rate of S-100 an… possibly also that of other brain proteins with similar physiological functions.

A necessary step for estimating the synthesis of specific proteins was the establishment of … consistently reproducible system for the *in vitro* incorporation at a high rate of amino aci… into protein. In addition the amount of proteins labelled should be sufficient to perm…

Abbreviations. SDS, sodium dodecyl sulfate TCA, trichloroacetic acid



[Fig.] Conditions for the incorporation of ¹⁴C-leucine into protein by brain slices. Slices of 80 mg in 2 ml ... were incubated at 35° with 1.7 µM of ¹⁴C-L-leucine (specific activity 34 mCi/mmol). A) Incubation ... was for 3 h at increasing concentration of the amino acid mixture in ●—● Hanks medium ○—○ Krebs Ringer medium. The results are the mean of duplicate incubations. B) pH dependence of ... synthesis. Incubation of mice brain slices in Hanks medium for 5 h in the presence of an amino ... mixture, final concentration 0.28 mM. The results are the mean of duplicate incubations. C) As in A ... the time indicated and with an amino acid mixture, final concentration 0.28 mM. The results are mean ± S.E. of 7 independent expts. each run in duplicate.

Results

...teristics of the incorporation system. Brain slices from rats or mice were incubated ...her Hanks or Krebs Ringer medium. Using the small scale incubation system the ...ditions for maximal incorporation were studied in detail. The incorporation of ¹⁴C-leucine ... protein was enhanced by adding D-glucose to the system showing a broad optimum at ... mM and a decrease at higher concentrations of D-glucose. Addition of a mixture ...mino acids at low concentrations had no effect on the incorporating activity but in...sing amounts inhibited the system (Fig. 2 A). As compared with Hanks medium slices ... Krebs Ringer medium seemed more sensitive towards the amino acid mixture. The in...porating activity showed a pronounced optimum at pH 7.1 measured at 35° (Fig. 2 B). ...r incubation of slices for 5 h the pH decreased to 6.95. The incorporation of ¹⁴C-leucine ... protein continued for 4 h, and Krebs Ringer and Hanks medium were equally good in ...porting protein synthesis *in vitro* (Fig. 2 C). Under the conditions used no significant ...rences ...ere obtained in the amino acid incorporating activity between slices from rats ... mice.

It is known (Ziegler and Melchior 1955) that preincubation of rat liver slices stimulates ... rate of amino acid incorporation into protein. Results on the effect of preincubation ... rat brain slices are presented in Table II. A comparison was made between addition of ...-leucine at zero time and at 15 or 30 min preincubation. Radioactivity determined after ... min incubation with the radioactive amino acid present was highest after 15 min pre...incubation (310 counts) and lowest after 30 min preincubation (230 counts). However at a ...tal preincubation plus incubation time of 90 min the radioactivity values without pre...incubation (375 counts) and with 15 min preincubation (390 counts) were similar.

The uptake of ¹⁴C-leucine was determined by measuring the free intracellular pool of ...dioactive amino acids (Fig. 3). The maximal uptake was reached after 90 min at 35° while

TABLE I. Composition of the incubation media used.

Chemical	mM concentration of medium	
	Hanks	Krebs Ringer
NaCl	137.00	118.50
KCl	5.35	4.74
MgSO	0.41	1.19
$MgCl_2$	0.49	—
$CaCl_2$	1.26	.09
Na_2HPO	0.34	—
KH PO	0.44	1.19
D-glucose	53.00	47.00
Phenolred	0.05	0.05
$NaHCO$	13.90	2.49

of the homogenat TCA was added to a final concentration of 10 . After centrifugation for 10 min at 15 000 g 100 µl samples were pipetted on to filter paper discs and the radioactivity of free ¹⁴C-leucine was measured. Free intracellular ¹⁴C-leucine was then calculated after the ntercellular olume of the et weight had been determined w th ³H- nulin (Regier and Kafatos 1971).

(b) *Large scale incubation.* Slices (500 mg) obtained from brain of 1 or 2 rats or from 2 3 mice ere incubated. A 100 ml E-flask containing 10 ml of Hanks medium (Table I), pH 7 1 per 500 mg et eight was used. To this was added 23 µC of H L-amino acid mixture alternatively 23 µCi H-L-leucine and 23 µC H L valine plus the mixture of unlabelled amino acids (Paul 1970) the radioactive ones being omitted (*final concentration 0 27 mM*) *wer added. After 5 h incubation at 35 the slices were homogenized in the* incubation medium. Portions of 5 ml were sonicated f r 2 min each in an ultrasonic disintegrator 500 Watt Model (M.S.E., London, U K) at 1 A and centrif ged for 1 h t 105 000 g $(NH_4)_2SO$ at final saturation of 60 (0.390 g/ml) was added to the supernatant under stirring at 4 A pH of 8.3 was maintained by adding solid K_2CO_3. After 15 min the suspension was centrifuged for 7 min at 15 000 g The supernatant was dialyzed t 4 against 0.15 M NaCl with 4 changes during the first 2 h, followed by 14 h against elec tropboresis buffer (see below). The dialyzed solution was concentrated with Aquacide II or Minicon B 15 at 4 to 0.5–1 ml and analysed for protein. Radioactivity was determined as described bove *but instead of filterpaper discs* glass f ber filters were used. The counting efficiency for tritium was 35 %.

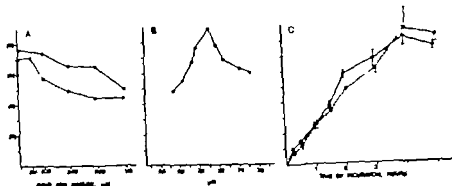
Polyacrylamide gel electrophoresis. This was essentially as described by Da id (1964) and Uyemura *et al.* (1971). The tubes for electrophoresis (120–72 mm) were filled w th the resolving 14 gel mixture to a height of 78 mm followed by a layer of 8 mm concentrating gel, pH 7 0 (62 mM Tris-HCl buffer 2.5 da 0.63 bisacrylamide, 0.5 ammonium persulfate and 0.058 TEMED 0.58 M sucrose) same concentrating gel but with pH 6.4. After polymerization the test solutio 0.6 mg of protein containing traces of bromophenol blue) was layered on t at 3 mA/tube until the dye had reached 1 cm from the bottom of the tube. T sisted of 60 mM Tris-base and 38.6 mM glycine, pH 8.6.

Radioactivity measurements of the gels. The gel was cut into 0.33 mm t them were placed into one vial. Soluene 350 (0.5 ml) was added, the for 3 h at 55 After cooling 5 ml of Brays solution (Bray 1960) was tion spectrometer at 8 efficiency A gel run n parallel was stain the protein pattern measured in a Joyce Loebl Microdensitome

T o-dimensional electrophoresis. After electrophoresis as direction as described by Hultin and Sjoqvist (1971) but

Immunological identification of S-100 protein. After poly for proteins. The area corresponding to the front band gels run in parallel. The protein was recovered electrop a cationic filter The double-diffusion technique of O immunologically

Analyses. Proteins were determined by the method o a standard. Radioactive leucine was identified by 2 plates hich were developed in n-butanol acetic acid



. 2. Conditions for the incorporation of ^{14}C-leucine into protein by brain slices. Slices of 80 mg in 2 ml dium were incubated at 35° with 1.7 µM of ^{14}C-L-leucine (specific activity 34 mCi/mmol). A) Incubation rat brain slices as for 3 h at increasing concentration of the amino acid mixture in ●—● Hanks medium ○---○ Krebs Ringer medium. The results are the mean of duplicate incubations. B) pH dependence of tein synthesis. Incubation of mice brain slices in Hanks medium for 5 h in the presence of an amino d mixture, final concentration 0.28 mM. The results are the mean of duplicate incubations. C) As in A t for the time indicated and with an amino acid mixture, final concentration 0.28 mM. The results are mean ± S.E. of 7 independent expts. each run in duplicate.

Results

aracteristics of the incorporation system. Brain slices from rats or mice were incubated either Hanks or Krebs Ringer medium. Using the small scale incubation system the nditions for maximal incorporation were studied in detail. The incorporation of ^{14}C-leucine to protein was enhanced by adding D-glucose to the system showing a broad optimum at 7 to 61 mM and a decrease at higher concentrations of D-glucose. Addition of a mixture amino acids at low concentrations had no effect on the incorporating activity but increasing amounts inhibited the system (Fig. 2 A). As compared with Hanks medium slices Krebs Ringer medium seemed more sensitive towards the amino acid mixture. The incorporating activity showed pronounced optimum at pH 7.1 measured at 35° (Fig. 2 B). ter incubation of slices for 5 h the pH decreased to 6.95. The incorporation of ^{14}C-leucine to protein continued for 4 h, and Krebs Ringer and Hanks medium were equally good in pporting protein synthesis in vitro (Fig. 2 C). Under the conditions used no significant fferences were obtained in the amino acid incorporating activity between slices from rats d mice.

It is known (Ziegler and Melchior 1955) that preincubation of rat liver slices stimulates the rate of amino acid incorporation into protein. Results on the effect of preincubation of rat brain slices are presented in Table II. A comparison was made between addition of ^{14}C-leucine at zero time and at 15 or 30 min preincubation. Radioactivity determined after min incubation with the radioactive amino acid present was highest after 15 min preincubation (310 counts) and lowest after 30 min preincubation (230 counts). However at a tal preincubation plus incubation time of 90 min the radioactivity values without preincubation (375 counts) and with 15 min preincubation (390 counts) were similar.

The uptake of ^{14}C-leucine was determined by measuring the free intracellular pool of radioactive amino acids (Fig. 3). The maximal uptake was reached after 90 min at 35° while

TABLE I Composition of the incubation media used.

Chemical	mM concentration of medium	
	Hanks	Krebs Ringer
NaCl	137 00	118.50
KCl	5 35	4.74
MgSO	0.41	1 19
$MgCl_2$	0.49	—
$CaCl_2$	1.26	2.09
Na_2HPO	0.34	—
KH PO	0.44	1 19
D-glucose	53.00	47 00
Phenolred	0.05	0.05
NaHCO	13 90	2.49

of the homogenate TCA was added to a final concentration of 10 %. After centrifugation for 10 m 15 000 g 100 / l samples were pipetted on to filter paper discs and the radioacti ty of free ^{14}C-le was measured Free intracellular ^{14}C leucine was then calculated after the intercellular volume of the weight had been determined w th H- nulin (Regier and Kafatos 1971).

(b) *Large scale incubation.* Slices (500 mg) obtained from brain of 1 or 2 rats or from 2 3 mice incubated. A 100 ml E flask contain g 10 ml of Hanks medium (Table I), pH 7 1 per 500 mg et c was used. T this was added 23 / Ci of ^{3}H L-amino acid mixture alternatively 23 μCi H L-leucine 23 μCi H L valine plus the mi ture of unlabelled amino acids (Paul 1970) (the radioacti e ones being om (final concentration 0.27 mM) were added After 3 h incubation t 35° the slices were homogenized in incubation medium. Portions of 5 ml were sonicated f 2 min each n n ultrasonic disintegrator 500 W Model (M.S.E. London, U K) at 1 A and centrif ged for 1 h at 105 000 g (NH $)_2$SO at a final saturat of 60 % (0.390 g/ml) was dded to the supernatant under stirring at 4 A pH of 8.3 was maintained adding solid K_2CO_3. After 15 min the suspension was centrifuged fo 7 mi t 15 000 g The supernat was dialyzed at 4 against 0.15 M N Cl with 4 changes during the first 2 h, followed by 14 h against el trophoresis buffer (see below) The dialyzed solution was concentrated with Aquacide II or Minicon B at 4 to 0.5–1 ml and analysed for protei Radioactivity was determined as described bo but instead filterpaper discs glass f ber filters were used. The cou ting efficiency for tritium was 35 %.

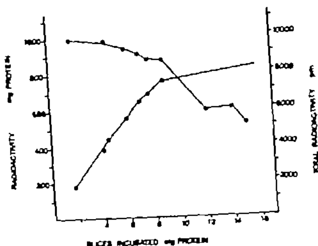
Polyacrylamide gel electrophoresis. This was essentially as described by David (1964) and Uyemura et (1971). The tubes for electrophoresis (120–72 mm) were f lled with the resolv g 14 gel mixture to hei of 78 mm followed by a layer of 8 mm concentrating gel, pH 7 0 (62 mM Tris-HCl buffer 2.5 acrylamid 0.63 % bisacrylamide, 0.5 % ammonium persulfate and 0 058 TEMED 0.58 M sucrose) and 8 mm of t same concentrating gel but with pH 6 4 After polymerization the test solution in 0.25 M sucrose (0 0.6 mg of protein containing traces of bromophenol blue) was layered on to the gel. Electrophoresi at 3 mA/tube until the dye had reached 1 cm from the bottom of the tube. The electrophoresis buffer co sisted of 60 mM Tris-base and 38.6 mM glycine, pH 8 6.

Radioactivity measurements of the gels. The gel was cut into 0.33 mm thick slices with a gel-slicer nd 3 them were placed into one vial. Soluene-350 (0.5 ml) was added, the vial covered with its cap and heate for 3 h at 55 After cooling 5 ml of Brays solution (Bray 1960) was added before counting in the sci tilla tion spectrometer at 28 % efficiency A gel run in parallel was tained w th Amidoblack 10 B, destained an the protein pattern measured Joyce Loebl Microdensitometer Model E 12 MK III

Two-dimensional electrophoresis. After electrophoresis as described bo the gels ere run in a secon direction as described by Hultin and Sjöqvist (1971) but in 14 acrylamide.

Immunological identification f S-100 protein. After polyacrylamide gel electrophoresis one gel was staine for proteins. The area corresponding to the front band of the stained gel was excised from the unstaine gels run in parallel. The protein was recovered electrophoretically (Kaltschmidt and Wittmann 1969) usin a cationic filter The double-diffusion technique of Ouchterlony (1948) was used to identify the protein immunologically

Analyses Protein were determined by the method of Lowry et al (1951) using bovine serum albumin as standard. Radioactive leucine was identified by 2-dimensional thi layer chromatography on silicagel plates which were developed i n-butanol acetic acid H_2O (60 20 20, / /v) and phenol H_2O (75:25,)



Correlation between of slices incubated and incorporated into protein amounts of slices wet weight, presented as mg protein) were under conditions de- Fig. 2 B. ●—● ^{14}C incorporated per mg O—O total incorporation into protein. The the mean of 2 inde- expts.

...lled leucine gave a strong positive reaction with ninhydrine where the radioactive ... as recovered. The results show that metabolic conversion of ^{14}C-leucine was neg-

In experiments described so far the final incubation was 2 ml containing slices of 80 mg ...ight, which corresponds to 7–8 mg of protein. As illustrated in Fig. 4 the specific ...poration of ^{14}C-leucine per mg protein was fairly constant up to 9 mg of total protein thereafter decreased with increasing concentrations. On the other hand, there was a ...nuous increase in total amount of ^{14}C-leucine incorporated with increasing amounts ...es which levelled off at 160 mg wet weight (14–16 mg of protein). Thus, with 80 mg ...es present in 2 ml medium, not only a high specific incorporation, but also a high total ...rporation was obtained.

...corporation of H-amino acids into acidic proteins. In order to obtain a sufficient amount ...otein for isolation of the acidic brain proteins the large scale incubation system was used. ...minary experiments had shown that it was difficult to elute after electrophoresis the ...oactive proteins from the 14% polyacrylamide gel and to make them available for liquid ...tillation counting at a high yield. A 70% recovery of the radioactivity present in the gels ...obtained after treatment of 0.3 mm thick slices with Soluene 350 at 55°

After incubation of slices with H-amino acids acidic proteins were isolated by $(NH_4)_2SO$ ...ctionation and electrophoresis on polyacrylamide gel. Fig. 5 shows the pattern of the ...ne protein from mice together with the radioactivity distribution. Fig. 5 A shows the ...oactivity distribution after extraction of the unstained gel with Soluene 350. Fig. 5 B ...er extraction of the stained gel with Soluene 350. The results show that extraction of ...dioactivity from the gels after staining and destaining resulted in loss of radioactive proteins ...hich had not been sufficiently fixed by the staining procedure.

The pattern of rat brain proteins and radioactivity distribution are shown in Fig. 6. The ...100 protein together with other acidic proteins were well separated from the bulk of soluble ...oteins. In the case of the S-100 protein containing band the radioactivity amounted to ...0.6–0.2% that of the total soluble proteins.

TABLE II Effect of preincubation of slices on the incorporation of ¹⁴C-leucine into protein. Rat brain slices were incubated under the conditions described in Fig. 2 B. ¹⁴C-leucine was added at zero time or at 15 or 30 min after the start of incubation. The total incubation time of the slices as indicated 60, 75 or 90 min. The results are the mean of 2 independent expts.

¹⁴C-leucine added	Radioactivity counts/min/mg protein		
	at 60 min	70 min	90 min
zero time	270	310	375
after 15 min	265	310	390
after 30 min	150	180	230

the synthesis of the intracellular proteins continued to increase for another 150 min. Similar results were obtained with slices from rats and mice. The amount of intracellular radioactive amino acid was calculated after the intercellular space had been determined with ³H-inulin (Reger and Kafatos 1971) The intercellular space was found to be 32.7 ± 1 6 µl per 100 mg of wet weight tissue.

It has been reported (Banker and Cotman 1971) that amino acids are metabolized in brain and thus not utilized as precursor for proteins. Therefore, the amount of free ¹⁴C-leucine present in the slices after 5 hours of incubation was determined by 2-dimensional thin layer chromatography 95% of the radioactivity applied to the origin was recovered in the area corresponding to the R_f values of leucine. These are 0 47 and 0 48 for the butanol and phenol system respectively (Randerath 1962) A control run in parallel and containing excess

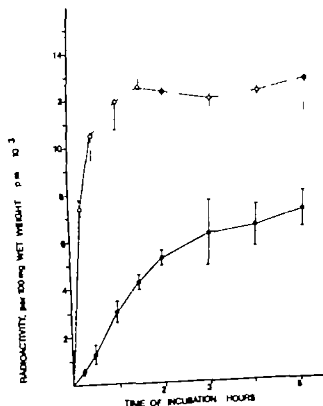
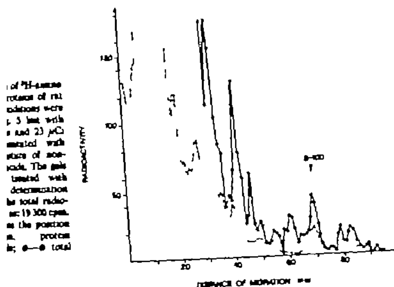


Fig. 3 Uptake of ¹⁴C L-leucine by brain slices Slices were incubated 0.23 µM ¹⁴C L-leucine (specific activity 250 mCi/mmol) for the time indicated. The amount of ¹⁴C-leucine present in tissue and that incorporated into protein was determined (see Material and Methods). The intercellular space was measured using ³H-inulin and found to be 32.7± µl/100 mg wet weight. The amount of intracellular free ¹⁴C-leucine was calculated. The results are the mean ± S.E. of 3 independent experiments each run in duplicate. Radioactivity per 100 mg wet weight, of protein ●—● free intracellular ¹⁴C-leucine O—O



i of ³H-amino
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tion of S-100 protein. The band migrating in front contained S-100 protein
ed with an arrow in Fig. 5 and 6. S-100 protein was identified by its charac
phoretic mobility (Moore 1965, Moore and McGregor 1965). A purified
f S-100 protein coelectrophoresed with the front band and an increase in
ity was obtained. Another characteristic of S-100 protein is its solubility in
ed $(NH_4)_2SO_4$. As shown in Fig. 7 the fraction remaining in solution at 60%
ntained several separated acidic proteins which were almost absent at 100%
ble S-100 protein remained in solution at the high salt concentration. The
band seen after precipitation at 60% saturation of $(NH_4)_2SO_4$ and which is
t to the S-100 protein is presumably the 14-3-2 protein described by Hydén
1970). This protein fraction was insoluble at 100% $(NH_4)_2SO_4$.
band was further identified as containing S-100 protein by precipitation with
bodies to beef S-100 protein (Fig. 8).
bility remained that the S-100 protein of the fraction soluble at 60% $(NH_4)_2SO_4$
eparate from other proteins in the one-dimensional polyacrylamide gel electro-
has been shown (Haglid and Stavrou 1973) that the front band after electro-
10% polyacrylamide consists of more than a single protein. The additional
a glycoprotein soluble in 50% methanol (Ramirez et al. 1974, Haglid et al. 1975).
a 2-dimensional electrophoresis in polyacrylamide was run to analyse the proteins
he second electrophoresis contained SDS to assure good separation under these
. The S-100 protein containing band of the first electrophoresis resolved into a
t. This is often observed after a 1-dimensional electrophoresis in a continuous
em (Haglid and Stavrou 1973). To remove the glycoprotein one gel slab was soaked
ethanol for 4 h before being stained with Amidoblack (Fig. 9 B). No convincing
as observed between the treated and untreated gel slabs (Fig. 9 A and B).

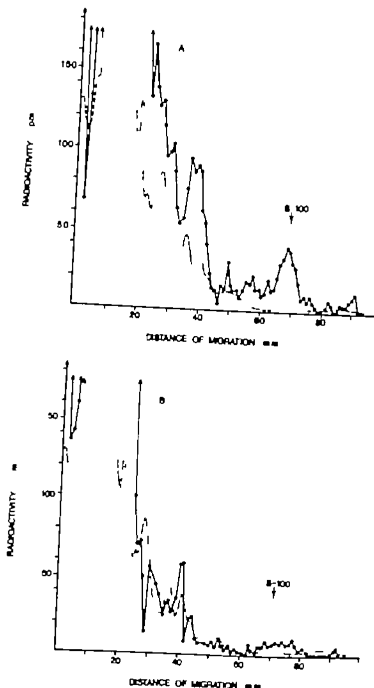


Fig. 5 Incorporation of H-amino acids into soluble proteins of mice brain. Slices (500 mg) were incubated in 10 ml of Hanks medium with 23 µCi of the H-amino acid mixture. After incubation of 5 h at 35° the slices were processed, the sol ble proteins fractionated, concentrated w th Minicon B 15 and subjected to polyacrylamide gel electrophoresis. A) The gels were sliced and treated w th Soluene-350 before determination of radioactivity B) The gels were stained and destained before treatment with Soluene-350 and radioactivity determination. The total radioactivity recovered was A) 17 900 cpm, B) 9 000 cpm. The arrow indicates the position of the S-100 protein. – protein pattern of the gels ●—● total radioactivity



Fig. 8. Two-dimensional electrophoretic pattern of brain proteins soluble at 60 (NH$_4$)$_2$SO$_4$. The arrow shows S-100 protein. (a) The gel was stained and destained directly after electrophoresis. (b) The gel was fixed in 50% methanol before staining and destaining.

with isotachophoresis rather than with conventional disc electrophoresis. Under these conditions the glycoprotein may be retarded differently from that of S-100 protein. The incorporation of amino acids into the S-100 protein containing band is relatively low as compared with the synthesis of serum albumin by rat liver slices (Campbell and Stone 1957). This can be explained partly by the low concentration of this protein present in the brain (Hydén and Lange 1970). The half life of S-100 protein has been reported to be 16 days (Cicero and Moore 1970), 3 days (Herschmann 1971) or 1 day and less (McEwen and Hydén 1966). However the low incorporation of radioactive amino acids would indicate a long half-life.

The radioactivity incorporated into the band containing S-100 protein was between 0.06 and 0.2 that of the total soluble proteins. This is consistent with the findings by other workers in vivo (0.1–0.5%, Zomzely-Neurath *et al.* 1972) and in cultures of human glial cells (0.9% ... Herschmann 1971).

The incorporation of radioactive amino acids into acidic proteins other than S-100 was high. The nature of these proteins has not been determined. The peak next to S-100 protein is probably the 14-3-2 protein earlier identified as protein 4 and 5, and described by Hydén and Lange (1970) as being specifically related to learning.

The radioactivity values were obtained after ultrasonication of the incubated homogenized slices. Ultrasonication is known to release newly synthesized soluble proteins from the microsomal fraction (von der Decken and Campbell 1964) and, thereby to increase the concentration of the soluble proteins.

To obtain a high incorporation of amino acids into protein the pH of the incubation system, and the amount of slices per volume were found to be of greatest importance. The transport of ^{14}C-leucine into cells was not a limiting factor of the system. The uptake was rapid and levelled off with time of incubation. Uptake of amino acids by rat brain slices has been studied in detail (Jones and McIlwain 1971). At least 30 min were required at 37° to reach equili...

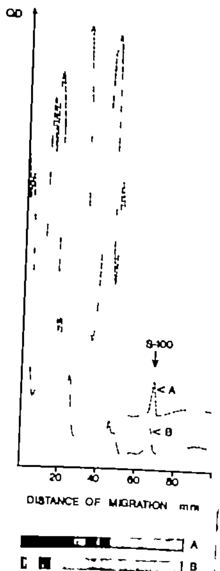


Fig. 7



Fig. 8

Fig. 7 Disc-electrophoretic patterns of soluble proteins from mouse brain. A) Soluble fraction obtained at 60% saturation of $(NH_4)_2SO_4$ B) Soluble fraction obtained at 100 saturation of $(NH_4)_2SO_4$. The amount of protein added to the electrophoresis tubes corresponded t 300 mg wet weight of tissue. The proteins were stained with Amidoblack 10 B, destained and the pattern measured in a microdensitometer

Fig. 8. Immunodiffusion of S-100 protein. The middle well contained a t bodies to beef S-100 protein the outer wells the antigen recovered electrophoretically from polyacrylamide gels. The protein concentration of the antigen was 0.35 mg/ml.

Discussion

In the present studies the synthesis of soluble acidic proteins *in vitro* by slices from brain of rats and mice is clearly demonstrated. The proteins were separated by polyacrylamide gel electrophoresis. The most acidic band migrating in front was shown to contain S-100 protein There is a possibility of a glycoprotein being present in the same band (Haglid and Stavrou, 1973) although treatment of the gels with 50% methanol did not seem to remove any protein. The type of the 1-dimensional electrophoresis used here with 2 spacergels is to be compared

Glassman, E., The biochemistry of learning: An evaluation of the role of RNA and protein. *Ann. Rev. Biochem.* 1969 38, 605–646.

Haglid, K. G. and D. Stavrou, Water-soluble and pentanol-extractable proteins in human brain normal tissue and human brain tumours, with special reference to S-100 protein. *J. Neurochem.* 1973, 20, 1523–1532.

Haglid, K. G., L. Rönnbäck and D. Stavrou, Purification of soluble glycoproteins from human nervous tissue and liver. *J. Neurochem.* 1975, 24, 1053–1057

Hazel, J. H. and R. E. Wallace, Relation of oxygen and temperature in the preservation of tissue by refrigeration. *Proc. Soc. exp. Biol. (N.Y.)* 1949 71 196–200.

Herschman, H. R. Synthesis and degradation of a brain-specific protein (S-100 protein) by clonal cultured human glial cells. *J. biol. Chem.* 1971 246, 7569–7571.

Hiller, T. and A. Schrier, Analogies in protein pattern and conformation among histones from different classes of vertebrates. *Comp. Biochem. Physiol.* 1971 40 B, 1011 1027

Hydén, H. and P. W. Lange, Correlation of the S-100 brain protein with behaviour. *Exp. Cell Res.* 1970. 62 125–132.

Jones, D. A. and H. McIlwain, Amino acid distribution and incorporation into proteins in isolated, electrically-stimulated cerebral tissues. *J. Neurochem.* 1971 18, 41 58.

Kaltschmidt, E. and H. G. Wittmann, Ribosomal proteins: VI Preparative polyacrylamide gel electrophoresis as applied to the isolation of ribosomal proteins. *Analyt. Biochem.* 1969, 30, 132 141

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall, Protein measurement with the Folin phenol reagent. *J. biol. Chem.* 1951 193, 265–275.

Mans, R. J. and G. D. Novelli, Measurement of the incorporation of radioactive amino acids into protein by a filter paper disc method. *Arch. Biochem.* 1961 94, 48–53.

McEwen, B. S. and H. Hydén, A study of specific brain proteins on the semi-micro scale. *J. Neurochem.* 1966 13, 823–833.

McIlwain, H. and H. L. Buddle, Techniques in tissue metabolism. I. A mechanical chopper. *Biochem. J.* 1953 53, 412–420

Moore, B. W. A soluble protein characteristic of the nervous system. *Biochem. biophys. Res. Commun.* 1965 19, 739–744.

Moore, B. W. and D. McGregor, Chromatographic and electrophoretic fractionation of soluble proteins of brain and liver. *J. biol. Chem.* 1965, 240 1647–1654.

Ouchterlony, Ö. *In Handbook of Experimental Immunology.* Weir, D. M. (ed.) Blackwell Scientific Publications Oxford. 1967 655–706

Paul, J., In *Cell and Tissue Culture.* E. & S. Livingstone, Edinburgh and London 1970 104.

Lomez, G., K. G. Haglid, B. Karlsson and L. Rönnbäck. Purification and characterization of soluble glycoprotein from rat brain (GM 50-C) *FEBS Letters* 1974 38, 143–146.

Randerath, K., In *Dünnschicht-Chromatographie.* Verlag Chemie G.m.b.H., Weinheim/Bergstr. 1962 70–80.

Rieke, J. C. and F. C. Kafatos, Microtechnique for determining the specific activity of radioactive intracellular leucine and applications to in vivo studies of protein synthesis. *J. biol. Chem.* 1971 246, 6480–6482.

Sellers, H. and A. Lajtha, The distribution of amino acids, Na and K from surface to centre in incubated slices of mouse brain. *J. Neurochem.* 1974, 22 977–985

Uyemura, K., G. Vincendon, G. Gombos and P. Mandel, Purification and some properties of S-100 protein fractions from sheep and pig brains. *J. Neurochem.* 1971 18 429–438

Vaz, A. and A. von der Decken, Incorporation of radioactive amino acids into proteins of brain slices from rat and mouse. *IXth Intern. Congr. Biochem. Stockholm Abstracts* 1973 154

Zamecnik, D. M. and J. B. Hultin, Activation of the amino acid-incorporating system of rat liver slices upon incubation. *J. biol. Chem.* 1955 217 569–577

Zomzely-Neurath, C., C. York and B. W. Moore, Synthesis of a brain-specific protein (S-100 protein) in a homologous cell-free system programmed with cerebral polysomal messenger RNA. *Proc. nat. Acad. Sci. (Wash.)* 1972 69 2326–2330

Zuckerman, J., H. Herschman and L. Levine, Appearance of a brain specific antigen (The S-100 protein) during human foetal development. *J. Neurochem.* 1970 17 247 251

incorporation of radioactive amino acids into protein continues after that equilibrium tween medium and slices is reached (Dunlop, van Elden and Lajtha 1974). Under the co tions used in the present investigation protein synthesis continued for another 150

It has been reported (Folbergrová 1966) that an amino acid mixture added to a s system decreased rather than increased the incorporation of radioactive amino acids protein. In the present results an inhibition was observed at higher concentrations of r radioactive amino acids. Metabolic conversion of radioactive amino acids being hig vivo in brain (Banker and Cotman 1971) was shown to be low in cerebral slices after 120 of incubation (Sershen and Lajtha 1974). A similar low conversion was obtained here w. ^{14}C leucine was analyzed after 5 hours of incubation.

The present method permits processing of amounts of brain tissue adequate for investi tion of the synthesis of specific acidic proteins. It can be applied to studies of changes in synthesis of these proteins caused by alterations in the physiological condition of the anim Changes in the environment of the animal have been shown to affect protein- and RN metabolism in the brain (von der Decken and Wroński 1971 von der Decken and Anders 1972) Further it is known that both these processes are involved in brain functions such learning and memory (for ref., see Glassman 1969)

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References

BANKER, G and C. W COTMAN, Characteristics of different amino acids as protein precursors in mou brain advantages of certain carboxyl-labeled amino acids. *Arch. Biochem.* 1971 *142*. 565-573.

BRAY H G A simple efficient liquid scintillato for counting aqueous solutions in a liquid scintillatio counter *Analyt Biochem.* 1960. *1* 279–285.

CAMPBELL, P N and N E. STONE, The synthesis of serum album and tissue proteins in slices of rat liv and liver tumor *Biochem. J* 1957 *66* 19–31

CICERO, T J and B. W MOORE, T rnover of the brai specific protein S-100. *Science* 1970. *169* 1333–133

COHEN, P P In *Manometric Techniques and Tissue Metabolism* Umbreit, W W B rris, R. H and Stauffe J F (eds). Burgess Publishing Co., Minneapolis 15 Minn., U.S A. 1951 119

DAVIS, B J Discelectrophoresis II Method and application to human serum proteins. *Ann. N Y Acad. Sc* 1964. *121* 404–427

VON DER DECKEN A Modification of the n v tro amino acid incorporation capacity of rat liver after vivo and in vitro treatments. *Eur J Biochem.* 1968 *4* 87 94

VON DER DECKEN, A. and P N CAMPBELL, The effect of ultrasonic vibrations on the protein-synthesizin activity of microsome preparations from rat li er *Biochem J* 1964 *91* 195–201

VON DER DECKEN, A. and A. WROŃSKI, Protein synthesis in vitro in rat brain after short-term protei starvation and refeeding. *J Neurochem.* 1971 *18* 2383–2388

VON DER DECKEN, A. and G M ANDERSSON, Effect of protein take DNA-dependent RNA polymera activity and protein synthesis in vitro in rat liver and brain *Nut Rep Intern.* 1972. *5* 413–419

DEROBERTIS, E., Ultrastructure and cytochemistry of the synaptic region. *Science* 1967 *156*. 907 914.

DUNLOP D S., W VAN ELDEN and A. LAJTHA, Measurements f rates of protein synthesis in rat brai slices. *J Neurochem.* 1974. 22. 821–830.

FOLBERGROVÁ, J Incorporation of labelled amino acids nto protei of brain cortex slices in vitro in th presence of other non-radioacti e amino acids. *J Neurochem.* 1966. *13* 553–56..

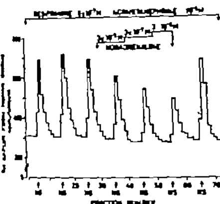


Fig. 1.

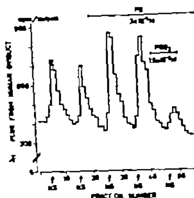


Fig. 2.

Fig. 1. Superfused human oviduct, preloaded with H-NA. Effect of NA on the outflow of tracer in response to transmural stimulation (NS), 150 pulses at 5 Hz. Time in min – fraction numbers.

Fig. 2. Superfused human oviduct, preloaded with ^{3}H-NA. Effects of phenoxybenzamine (PBA) and prostaglandin E_2 (PGE_2) on the outflow of tracer in response to transmural stimulation (NS), 150 pulses at 5 Hz. Time in min – fraction numbers.

transmitter release in the presence of uptake blockers suggest activities on presynaptic receptors.

Isoprenaline (4.7 10^{-8} 2.4 10^{-} M) produced inconsistent effects on evoked release of ^{3}H-NA, when the above experimental design was used (presence of uptake blockers and stimulation at 5 Hz). Since the lack of effect might be due to the presumed presynaptic β-receptors already being saturated, further experiments were therefore performed according to the schedule introduced by Langer (1975), namely low frequency stimulation (1 Hz, 60 pulses), no uptake blockade, and low doses of isoprenaline (4.7–9.5 10^{-8} M). Using this procedure, isoprenaline consistently enhanced the evoked release of H–NA (Fig. 3). The calculated increase by isoprenaline (0.5 10^{-8} M) of H–NA release resulting from transmural stimulation was 48.0 ± 15.1% (mean ± S.E., 6 expts, $p < 0.05$). The enhancing effect of this dose of isoprenaline was reduced to 6.3 ± 4.2% in the presence of propranolol (7 10^{-8} M). In most cases propranolol itself had no effect on evoked release of H–NA, although in some cases it caused a slight to moderate inhibition. Enhancement of evoked transmitter release by isoprenaline, the effect of which is annulled by propranolol, suggests activation of presynaptic β-receptors operating at low catecholamine levels.

During recent years data have accumulated indicating that NA release from adrenergic nerve terminals, in many tissues may be subject to feed back control by PGE (Hedqvist 1976) and by presynaptic α- and β-adrenoceptors (Starke 1972, Enero *et al.* 1972, Langer 1975, Sjöstrand and Brundin 1975). The present study and a previous report (Moawad *et al.* 1975) provide circumstantial evidence to show that NA release from the adrenergic nerves of the human oviduct may be controlled by any of these three mechanisms. If present *in vivo* two of them should be considered depressant and operate through activation of PGE and presynaptic α-adrenoceptors respectively and the third to mediate positive feed back via activation of presynaptic β-receptors. It is in addition conceivable that the presynaptic

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Presynaptic α- and β-adrenoceptor Mediated Control of Noradrenaline Release in Human Oviduct

By

P Hedqvist and A Moawad

In the human oviduct, which is richly innervated by adrenergic nerve fibres, particula[rly] in the isthmus, muscular effects of secreted noradrenaline (NA) are mediated by excitat[ory] α- and inhibitory β-adrenoceptors (*Owman et al* 1967 Rosenblum and Stein 1966). T[he] relative activities of these receptors appear to be under hormonal control since estro[gen] predominance enhances α-adrenoceptor activity while progesterone elevates that of [the] β-adrenoceptors (Moawad and Kim 1974).

In the search for presynaptic mechanisms controlling adrenergic transmission in the hum[an] oviduct, it has recently been shown that NA release by nerve activity is effectively suppres[sed] by prostaglandin E_2 (PGE_2) (Moawad *et al* 1975) In the present study we wish to rep[ort] data indicating that α and β-adrenoceptor agonists may negatively respectively positive[ly] affect the release of NA in this organ.

The isthmic part of h man o iducts, 1½ 2 cm length, was preincubated for 1 h n Tyrode contain[ing] 2.5 μCi/ml H-(−)-NA (spec. act 3.8 Ci/mmol, N E.N). It was then mounted in a 2 ml bath and superfused with Tyrode at a rate of 1 5 ml/min. The composition of the Tyrode was (conc. in mM): N[aCl] 136.7 KCl 2.7 $CaCl_2$ 1.8 $MgCl_2$ 1 0 $NaHCO_3$ 11 9 NaH_2PO_4 0 4 glucose 5.5, ascorbic acid 0.1 and was kept at 37°C and was gassed w th 5% CO_2 in O_2. The organ was transmurally stimulated by means [of] platinum electrodes in the wall f the bath and Grass S4 stimulator delivering trains of biphasic pul[ses] (1 5 Hz, 1 ms, supramaximal voltage) at 10–15 min intervals The superfusate was divided into one [min] samples and the radioactivity was determined by counting 0 5–1 ml aliquots in a Packard scintillat[ion] spectrometer using 10 ml Instagel as counti g medium. Quenching was monitored by [ex]ternal standar[d]. It has previously been shown that the bulk of tracer released by transmural stimulation consists of un[changed] H NA (Moawad [e]t al 1975).

In the presence of desipramine (5 10− M) and normetanephrine (10^{-4} M), administer[ed] in order to prevent uptake and uptake₂, NA dose-dependently and reversibly inhibited t[he] release of H–NA evoked by transmural stimulation (5 Hz, 150 pulses) (Fig. 1). The pe[r]centage inhibition of H NA release by NA 3 10^{-4} M and 3 10 M was 25 0 ± 7.8 a[nd] 39.3 ± 7 9 respectively (means ± S.E. 4 expts, p-values 0 05) On the other hand phenox[y]benzamine (PBA), 3 10^{-4} M approximately caused a 3-fold increase of evoked H N[A] release (Fig. 2) Notably the inhibition by PGE_2 of evoked H–NA release was marked[ly] reinforced in the presence of PBA. Inhibition by NA and enhancement by PBA of evok[ed]

Abstracts from Meeting of the Scandinavian Physiological Society in Copenhagen 25–26 April 1975

COMMUNICATIONS

C 1

Prostaglandins and Post-Ischemic Muscular Vasodilation

By Å. Wennmalm and Å. Kilbom. *Department of Clinical Physiology Karolinska Institute Serafimer Hospital, 112 83 Stockholm Sweden*

Prostaglandins of the E series (PGE) are powerful vasodilators. Recently it has been shown that hypoxia is a potent stimulus of PGE synthesis in a mammalian heart muscle (1) and that PGEs are possible mediators of reactive hyperemia (2) We have tested the hypothesis that endogenous PGEs take part in the vasodilation, and thus hyperemia, following arterial occlusion of the human forearm. Healthy volunteers, five men and five women, took part in the study Forearm blood flow was measured using venous occlusion plethysmography Muscle blood samples for PGE analysis were obtained through a catheter inserted in a medical cubital vein. The content of PGE in plasma samples was absorbed on Amberlite XAD-2 and further purified using conventional lipid extraction procedures. Quantitative and qualitative analysis of the purified plasma extracts was performed by using bioassay and thin layer chromatography

Each subject was studied twice, with 1 h interval. Initially the subject rested comfortably in a supine position, and the arterial blood flow was measured repeatedly after five minutes. Following this, arterial occlusion was applied with a pneumatic cuff on the upper arm. After five minutes, occlusion was released and the blood flow measured repeatedly during the following 2½ min. Blood samples for PGE analysis were collected at rest and during the first 2½ min after ischemia. Subsequently 100 mg of indomethacin (Indomée®-Merck, Sharp and Dohme) was administered rectally and after one hour the procedures of blood flow measurements and blood sampling were repeated.

The mean resting arterial blood flow was 3 7±0.5 (mean ± S E. n=10) ml/100 g tissue/min. No sex difference was obtained nor was the resting blood flow influenced by indomethacin. Following 5 min of arterial occlusion, marked increase in the blood flow was observed, both prior to and following administration of indomethacin. The concentration of PGE increased after ischemia. However the duration and the magnitude of the hyperemia was significantly decreased in the presence of indomethacin. In parallel the PGE concentration after ischemia was lower after indomethacin treatment.

Since administration of indomethacin in parallel decreased the magnitude of the reactive hyperemia and the concomitant release of PGE into the vein from the ischemic tissue we conclude that endogenous PGE, formed during tissue ischemia, take part in the development of muscular reactive hyperemia

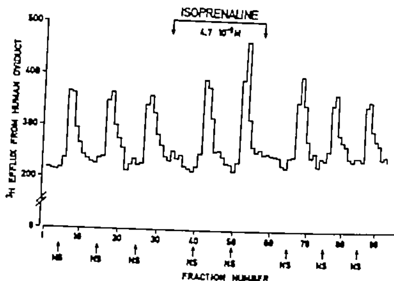


Fig. 3 Superfused human oviduct, preloaded w th H NA. Effect of isoprenaline on the release of tr in response to transmural stimulation (NS), 60 pulses at 1 Hz. Time in min = fraction numbers.

activities of PGE and adrenoceptors are themselves subject to variation, due to the hormo conditions, as are their activities at the effector cell level (Moawad and Kim 1974, Moaw *et al.* 1975).

Supported by grants from the Swedish Medical Research Council, project No 04X-4342.

References

ENERO, M A., S. Z. LANGER, R P ROTHLIN and F J E. STEFANO, Role of the α-adrenoceptor in regulat noradrenaline overflow by nerve stimulation *Brit J Pharmacol* 197_ *44* 672–688.

HEDQVIST P Prostaglandin action on transmitter release at adrenergic neuroeffector junctions. In *Advanc in Prostaglandin and Thromboxane R search*, Vol. _, eds B Samuelsson and R. Paoletti, pp. 357–36 Raven Press, New York 1976.

LANGER, S. Z., Prejunctional regulatory mechanisms for noradrenaline release elicited by nerve stimulation In *Chemical Tools in Catecholamine Research* ed T Malmfors, North-Holland Publishing Co., Amster dam. 1975 (in press).

MOAWAD, A. H and M KIM, Plasma sex steroid levels and the in tro response of the isthmic portion o human oviduct to catecholamines. *Gynec Inv st* 1974. *5* 19

MOAWAD A., P HEDQVIST and M BYGDEMAN Noradrenaline release follow g nerve stimulation and it mod fication by prostaglandin E_2 in human and rabbit oviduct *A t phy iol scand* 1975. *95* 142 144.

OWMAN Ch E. ROSENGREN and N O SJOBERG Adrenergic nervation of the human female reproductiv organs, a histochemical and chemical investigation *Obstet G necol* 1967 *30*. 763–773.

ROSENBLUM, L and A. A. STEIN, Autonomic responses of the circular muscles of the isolated human Fallo- pian tube. *Amer J Physiol.* 1966. *210*. 1127 1129

STARKE, K., Influence of extracellular noradrenaline on the st mulation-evoked secretion of noradrenalin from sympathetic nerves. evidence for an α-recepto -mediated feed-back inhibition of noradrenalin release. *Naunyn-Schmiedeberg s Arch. Pharmak* 1972 275 11 3

STJÄRNE, L. nd J BRUNDIN, Dual adrenoceptor-mediated control of oradrenaline secretion from human vasoconstrictor nerves. facilitation by β-receptors and h bition by α-receptors. *Acta ph iol. scand* 1975 *94* 139–141

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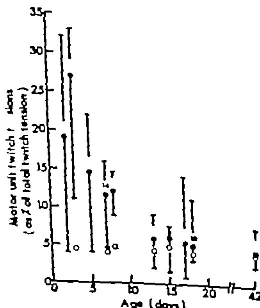
Large-scale Reduction in Motoneurone Peripheral Fields during Post-natal Development in the Rat

By M. C. Brown, J. K. S. Jansen and D. Van Essen. *Institute of Physiology, University of Oslo, Norway*

The end-plate potential (e.p.p.) in curarized newborn rat muscles shows 2 or more components that are elicited at different strengths of nerve stimulation (Redfern 1970). The following experiments on neonatal rat soleus muscles support the suggestion of Bennett and Pettigrew (1974) that a convergence of several axons to a single synaptic spot on each muscle fibre accounts for these multicomponent e.p.p. s. Only a single spot of cholinesterase was seen on individual stained and teased muscle fibres (confirming Zelená and Zelená 1966), but all components of the e.p.p. were reversibly increased in amplitude and time course after application of prostigmine. There was no sign of electrical coupling between adjacent muscle fibres impaled with separate microelectrodes. Finally, all components of the e.p.p. were equally depressed by iontophoretically applied dose of acetylcholine that desensitized receptors within about 50 μm of the acetylcholine pipette.

Multiple innervation disappeared during the second week after birth: after day 15 less than 5% of fibres had multiple inputs. We measured the number of motor units that could be isolated in ventral root filaments and the tension produced by single units. The number of motor units remained roughly constant at about 25 but (see Fig. 1) their mean twitch tension, expressed as percentage of the total twitch tension, declined dramatically between days 2–3 (mean 23%) and days 15–18 (mean 5.4%). A similar but smaller change

Fig. 1. Average twitch tensions of motor units (filled circles, ●, expressed as percentage of total twitch tension) and the range of sizes (vertical bars) found at various ages in the rat soleus muscle. Ventral root filaments containing between one and five motor units were stimulated while recording impulses from the soleus nerve and isometric twitch contractions from the muscle in an isolated preparation containing the muscle and its nerve dissected back to spinal roots L5 and L6 and bathed in oxygenated 10 mM Ca⁺⁺ Ringers solution buffered with Tris at room temperature. Open circles (O) give the average size of motor units that would be expected from the total number of units found if there were no multiple innervation. The number of units whose tension was measured individually is given for each muscle.



References

1 WENNMALM, Å. PHAM-HUU-CHANH and M. JUNSTAD *Nature* (Lond) (in press).
2. KENT K M et al. *Physiologist* 1973 *16* 361

C 2

Recordings of Single Unit Activity from Cutaneous Nerve Fibres in Man

By H HÄMÄLÄINEN T JÄRVILEHTO and P LAURINEN *Department of Ge* *Psychology University of Helsinki Finland*

Recording of single unit activity from cutaneous nerves in man presents a new app to the study of peripheral neural mechanisms underlying cutaneous sensations (Knit and Vållbo 1970) In the present work characteristics of single units in the human r nerve were studied with the purpose of determining the neural input necessary : touch sensation.

Tungsten microelectrodes (tip diameter 1–3 μm) were inserted into the radial ner the wrist by means of a micromanipulator Single unit potentials were amplified store magnetic tape and analyzed in a computer

Preliminary results are based on recordings from 20 single units Four units did respond to the manipulation of the skin. One of them was affected by movements of index finger and 3 units by cigarette smoking or deep breathing. The former unit h regular spontaneous discharge (mean of interval histogram $\bar{X}_1 = 137$ ms standard de tion, $s = 34$ ms) Pressing of the finger downwards caused ceasing of the discharge wh reappeared with lifting of the finger One unit fired in very regular burst of 10–40 impul separated by pauses of 1–4 s (within burst $\bar{X}_1 = 50$ ms $s = 20$ ms) Smoking of a cigar resulted in longer intervals both within and between the bursts and in marked reductio the number of impulses Deep breathing reduced firing frequency of 2 units one of th firing spontaneously in bursts (within burst $\bar{X}_1 = 19$ ms $s = 9$ ms inter-burst intervals. 0.5 s) the other having a regular discharge ($\bar{X}_1 = 56$ ms $s = 5$ ms) The latter units resem sympathetic units described by Delius *et al* (1972)

Sixteen units responded to mechanical stimulation of the skin According to the respon to sustained pressure 5 units could be classified as rapidly adapting and 11 as slow adapting (SA) ones Conduction velocities of 4 SA-units measured by applying electr pulses to receptive fields ranged from 12–32 m/s The thresholds of the SA-units for a fe impulses to appear ranged from 0 1–1.2 g, which corresponded well to the report threshold sensation. An average discharge of 10–20 imp/s however caused usually only weak touch sensation and for a moderate sensation impulse frequencies up to 40 imp/ were necessary The results suggest that a few impulses in a mechanoreceptive fibre ma suffice for detection of a tactile stimulus but a relatively large number of impulses i necessary for a distinct touch sensation.

References

DELIUS, W K E. HAGBARTH, A. HONGELL and B G WALLIN, *Acta physiol. scand* 1972. *84* 65–81
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C 3

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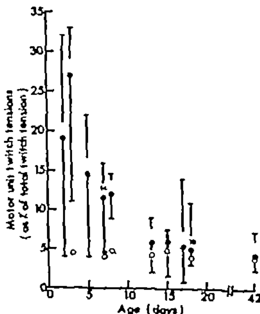


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was totally dependent on the presence of Mg^{2+} in the medium. There was no
the % of vasopressin in the filtrate either in a medium containing 1 mM EGTA
ald Ca^{2+}
ese experiments it would appear that the ATP-induced emptying of secretory
ments is, at least in part, an enzyme catalysed reaction.

References

, and D. B. Hope, *Biochem. J. 104* 1967 1082 1083.
. M. and W. W. Douglas, *Molecul. Pharmacol.* 1968. 4 531–540.

C 5

Control of Glycolysis and Oocyte Maturation in the Preovulatory Rat Ovarian

Hillensjö. *Department of Physiology University of Goteborg S eden*

ulatory surge of luteinizing hormone (LH) is known to induce several biocheml morphological transformations in preovulatory follicles. Thus the hormone he sterodogenic pattern, causes resumption of oocyte meiosis ("oocyte matura d induces changes in the follicular wall eventually leading to its rupture. The echanisms behind these effects are largely unknown. Concerning the induction of *aturation, several triggering factors within the follicle have been suggested.* In to teroids and cyclic AMP certa energy substrates such as pyruvate-lactate are mediators of this LH-effect (Lindner *t l* 1974).

r studies have shown that gonadotropins stimulate cyclic AMP formation and is in isolated preovulatory rat follicles (Lindner *t al* 1974 N isson 1974 Nilsson 74). The aim of the present study was to examine oocyte maturation and glycolysis ed preovulatory rat follicles xposed to gonadotropins *n vivo* or *in vitro*

ibertal rats were injected with 10 IU PMS when 30 days old leading to follicle ment and subsequent endogenous release of LH in the afternoon of day 32. Time of LH surge oocyte maturation and ovulation has been published (Hillensjö *et al* Rats were killed before and after the LH surge on day 32 the ovaries removed and latory follicles isolated by microdissection. The follicles were incubated in Krebs bicarbonate buffer containing 5.5 mM glucose and 1 % bovine serum albumin with thout hormone The follicles were mmersed in 0.5 ml medium in flasks gassed with –5 % CO_2. After incubation the lactate concentration in the medium was analysed atically *The oocytes were recovered for direct inspection using Nomarski in-* nce contrast microscopy The occurrence of germinal vesicle breakdown (GVB) t morphological sign of oocyte maturation was recorded

first group of rats were sacrificed between 9.00–13.00 the follicles isolated and ted *In control medium a linear lactate production was found during 0.5–6 h incuba-* The oocytes did not mature I presence of LH (NIH S18) 0.1–10 µg/ml or FSH S10) 1 10 µg/ml a pronounced stimulation of lactate production was seen. The ones also nduced GVB in the oocytes. After 2 h of incubation a small proportion of ocytes showed GVB whereas 90–100 % showed GVB after 4 h incubation.

has been reported in kittens (Bagust *et al* 1972) Our results suggest that the average of about 5 functional synapses on each muscle fibre shortly after birth and disappearance of multiple innervation occurs by means of a decline in the relative motor units with little change in their number

References

BAGUST J D M. LEWIS, J C LUCK and R. A. WESTERMANN, *J Physiol* 1972 224 35P
BENNETT M R. and A G PETTIGREW *J Physiol* 1974 *241* 515–545
LUBINSKA L and J ZELENÁ, *Nature* 1966 *210* 34–41
REDFERN P A *J Physiol* 1970 *209* 701–709

C 4

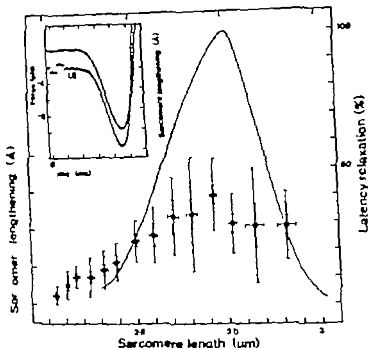
Adenosine Triphosphate-Induced Emptying of Isolated Bovine Neurohypophyseal Secre Granule Contents

By J T RUSSELL and N A THORN *Institute of Medical Physiology C Univer of Copenhagen Denmark*

It has been reported that ATP can induce release of vasopressin from otherwise st isolated neurosecretory granules (Poisner and Douglas 1968) However it is not cl whether this is due to membrane instability caused by ATP or an enzymatic effect.

A crude secretory granule fraction was obtained from bovine neurohypophyses (Fr *tion III* of Dean and Hope 1967). The granules were suspended in ? ml of incubati medium (composition (mM) KCl 124 5 NaCl 5 0 $CaCl_2$ 0 001 $MgCl_2$ 5 0 Tı *(hydroxymethyl)* methyl 2-aminoethane sulphonic acid ?0 0 pH 7 0) and aliquots we added to vessels with ?0 or 2 5 ml of incubation medium (final protein concentrati 200–300 μg/ml) and incubated at 37 or 5°C in a shaking water bath The reaction w stopped by filtering 2 0 ml aliquots of the reaction mixture through millipore filters (po *size 0 22* μm) The filtrates were passed through Whatman DE81 ion exchange discs t remove ATP The filtrate and an acetic acid (0 25 %) extract of the filter were assayed fo vasopressin activity The results were expressed as % of total vasopressin in the ? m aliquot appearing in the filtrate

In the absence of ATP approximately 10 % of the total vasopressin appeared in the filtrate even after 60 min of incubation. When ATP was added to the medium however more than 50 % of the hormone activity was found in the filtrate in 30 min. This effect of ATP was dose dependent showing a threshold concentration of 0 ? mM and a maximal effect at 1 0 mM ATP Inclusion of an ATP regeneration system further increased the % vasopressin in the filtrate to 80 This value was not different from the % hormone that could be released by vigorous sonication. Electron microscopic observation of the ATP-treated granules showed that they had totally lost their electron opaque cores but still possessed intact membranes The percentage appearing in the filtrate was significantly lower at 5°C than at 37°C (79.8+2.8 % at 37°C in 30 min, 51 9±4 1% at 5°C p=<0 001). N-ethylmaleimide, a known inhibitor of ATPase, blocked this ATP-induced emptying of granules at a conc of 2 mM The effect of ATP showed a pH optimum between 6–8.



…ean values of latency elongation shown with ±1 S.D. (thin bar) and ±1 S.E.M. (thick bar). …ure of latency relaxation indicated by continuous curve. Insert: Latency relaxation and …longation with stimulation waveform added

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References

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C 7

…stration of Tissue Hyperosmolality in the Tips of Intestinal Villi during Sodium …le Absorption

… Mats Jodal and Ove Lundgren. *Department of Physiology, University of …teborg, Sweden*

…e earlier proposed that a marked interstitial sodium concentration gradient along …gth of the cat intestinal villi was created during sodium absorption by a countercur… multiplication of sodium in the …lli, resulting in a high tissue osmolality in the tips of …lli (Haljamäe *et al.* 1973). This hyperosmolar compartment was proposed to be of … importance also for the absorption of water, particularly in the absence of/or …st … lumen-to-blood osmotic gradient.

The second group of rats were sacrificed between 18 00–19 00 OVB had then occ *in vivo* Lactate production of these follicles incubated in control medium were mai higher (3-fold) than of corresponding follicles of the morning group yet addition (0 1–10 $\mu g/ml$) resulted in a slight but significant increase

The results show that properly characterized PMS-treated prepubertal rats consti convenient experimental model for studies of oocyte maturation and related bioche phenomena. The finding that exogenous as well as endogenous gonadotropins in ad to the meiosis inducing action markedly enhanced follicle glycolysis suggests that actions might be related Further studies are needed in order to establish a casual rel ship between these two events.

References

HILLENSJÖ, T A BARNEA L. NILSSON H HERLITZ and K AHRÉN, *Endocrinology* 197· 1762–1766

LINDNER, H R. A. TSAFRIRI M E. LIEBERMAN U ZOR Y KOCH S BAUMINGER and A BARN *Recent Progress in Hormone Research* Ed R O. Greep A. P N Y 1974 30 79–138

NILSSON L. *Acta endocr* (Kbh) 1974 77 540–558

NILSSON L S. ROSBERG and K. AHRÉN, *Acta endocr* (Kbh) 1974 77 559–574

C 6

Early Mechanical Response in Isolated Frog Skeletal Muscle Fibre

By P HAUGEN and O STEN KNUDSEN *Department of Biophysics Universit Copenhagen Denmark*

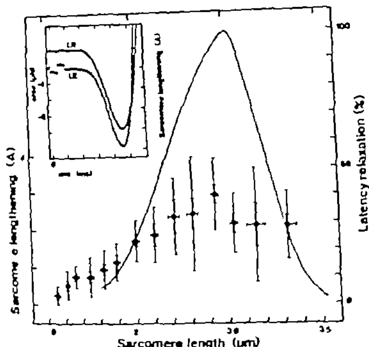
The latency relaxation and the latency elongation (the corresponding lengthening of sarcomeres) were investigated in single muscle fibres

The latency elongation was measured by a laser diffraction technique using ph electric difference detection to register small changes in the position of the first o spectrum resulting from the latency elongation Sarcomere length changes as small as 0 could be measured The fibre was stimulated transversely to ensure synchronou excitation

Latency relaxation was measurable at sarcomere lengths from 2.15 μm to 3.5–3 7 μm contrast to Mulieri s (1972) findings the amplitude of the tension drop passed throu sharp maximum which occurred at a sarcomere length of 3 0–3 1 μm Latency relaxa is dependent on overlap between thick and thin filaments The effect of the sarcom length on the time course agrees with the findings of Guld and Sten Knudsen (1960).

The latency elongation was not uniform along the fibre In some regions maxim lengthening occurred either before or simultaneously with the maximum tension drop other regions the sarcomeres did lengthen further before contraction and might even pulled further out by contraction of neighbouring sarcomeres In the range from 2 3 μm 3.0 μm the mean latency elongation increased by a factor of 3 while the latency relaxat increased 20 times

In completely slack fibres where no drop in tension could be detected latency elongat was observed down to 2.05 μm. Thus, the latency relaxation is caused by an act



[M]ean values of latency elongation shown with ±1 S.D. (thin bar) and ±1 S.E.M. (thick bar) [Si]ze of latency relaxation indicated by continuous curve. Insert: Latency relaxation and [el]ongation with stimulation waveform added

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C 7

[Demons]tration of Tissue Hyperosmolality in the Tips of Intestinal Villi during Sodium [Chlor]ide Absorption

[B]y MATS JODAL and OVE LUNDGREN. *Department of Physiology University [o]f Göteborg S[w]eden*

[It w]as earlier proposed that a marked interstitial sodium concentration gradient along [the le]ngth of the cat intestinal villi was created during sodium absorption by a countercur[rent] multiplication of sodium in the villi resulting in a high tissue osmolality in the tips of [the vi]lli (Haljamäe *et al.* 1973). This hyperosmolar compartment was proposed to be of [majo]r importance also for the absorption of water particularly in the absence of/or [agains]t a lumen-to-blood osmotic gradient

According to the countercurrent hypothesis a small increase of plasma sodium conc
ration in the subepithelial villus capillaries caused by active sodium absorption, resul
a crossdiffusion of sodium from the subepithelial network into the central arterial ve
or/and of water in the opposite direction. In the mentioned study this hypothesis
substantiated by demonstrating that the sodium content per unit weight tissue protein
3–4 times higher at the villous tip than at the base

To obtain a more direct measure tissue hyperosmolality has now been determine
cryoscopy During net water absorption from isotonic NaCl-glucose solution norm
blood perfused intestinal segments were suddenly frozen in liquid nitrogen or isopent
Intestinal slices 10 μm thick were cut at −20°C in a cryostate and the sections v
microscopically examined in a room at −10°C The temperature of the intestinal sect
was increased in a stepwise fashion and the temperature at which thawing occurre
different parts of the intestinal wall was determined The villus tips were clearly the
intestinal structures to thaw and a gradient of freezing point depression was observed a
the villi. The crypt sections thawed at the same temperature as the submucosa and
muscularis corresponding to an osmolality of slightly above 300 mOsm/kg In contrast
freezing point depression in the villous tips corresponded to a tissue osmolality of arc
1 200 mOsm/kg. Thus the obtained results are in agreement with the proposed counter
rent multiplication hypothesis

References

HALJAMÄE, H M JODAL and O LUNDGREN *Acta physiol scand* 1973 89 580–593

C 8

Osmolar Control of Extracellular and Plasma Volume in Hemorrhagic Hypotension

By JOHANNES JARHULT *Department of Physiology University of Lund Sweden*

The aim of the present investigation was to define blood osmolality changes dur
hemorrhagic hypotension their underlying mechanisms and their importance for fl
volume redistribution between different body compartments in hypovolemia. The exp
ments were performed on anesthetized cats exposed to standardized rapid bleeding to
constant arterial pressure level of 50 mm Hg

Hemorrhage caused a rapid and pronounced increase of arterial plasma osmolality whi
almost fully could be accounted for by a concomitant hyperglycemia The hemorrha
hyperglycemia was caused by glucose release from the liver in turn mediated via
different reflex mechanisms By direct sympathetic nervous influence on the liver
adrenergically released glucagon from the pancreas and by catecholamines emanati
from the adrenal medulla. Quantitative investigations of capillary fluid exchange in ske
tal muscle revealed a considerable transcapillary absorption of extravascular fluid into t
blood stream during the first hour of hemorrhagic hypotension. Such fluid transfer o
curred not only in the intact muscle region, but also after regional α-adrenergic blocka
and sympathectomy showing that mechanisms other than the previously known adrenerg
reflex resetting of the pre-/post-capillary resistance ratio contributed to the fluid a

ption. Critical analysis indicated that an osmotic mechanism, related to the arterial hyperosmolality (hyperglycemia), was responsible for the capillary fluid transfer in the sympathectomized muscle. Transcapillary absorption of extravascular fluid from skeletal muscle evoked by both these mechanisms is of great hemodynamic importance in hemorrhage insofar as it helps to restore plasma volume. Extrapolation of the present observations to man suggests that a total extravascular fluid volume of about 600 ml would be absorbed into the blood stream from all skeletal muscle tissue within the first hour of hemorrhagic hypotension. The osmotic mechanism would be responsible for at least 50 per cent of this effect.

It appears that the described osmolar fluid control is basically aimed at an overall regulation of extracellular fluid volume in hemorrhage causing a glucose-osmotic fluid (water) withdrawal from the intracellular compartment. The consequent dilution of protein in the interstitial space of the tissues can help to explain why the osmotically and finally absorbed protein-poor extravascular fluid from skeletal muscle is retained for considerable length of time within the circulatory system. The efficiency of this mechanism increased if the interstitial protein concentration is high before dilution. Recent studies have indicated that interstitial colloid-osmotic pressure indeed is significant and much higher than previously assumed (see Aukland 1973).

Reference

AUKLAND, K., Autoregulation of interstitial fluid volume. Edema-preventing mechanisms. *Scand. J. Clin. Lab. Invest.* 1973, *31* 247–254

C 9

Thermal Panting Reduces Oxygen Uptake in the Dik Dik, *Rhynchotragus kirkii*

By P P HOPPE, K. JOHANSEN G M. O MALOIY and V MUSEWE. *Department of Animal Physiology University of Nairobi K nya, and Department of Zoophysiology University of Aarhus Denmark*

study of oxygen uptake (VO_2) during heat stress in the Dik Dik, a small ruminant antelope (2.5–7 kg) is of special interest because of its high metabolic rate and labile core temperature. The animal inhabits semi-arid bush country in East Africa and encounters intense radiative heat. Its *practice of thermal panting has been reported earlier* (Maloiy 1973).

VO_2 was measured at thermoneutrality (Ta=22–27°C) and during heat stress (Ta=40–43°C) in a temperature controlled room. The animals were suspended in a harness and fitted with face masks during measurements. A Servomex O_2 analyzer gas flowmeters and pumps were used for the VO_2 measurements. Respiratory rate, rectal and skin temperatures w re continuously recorded. The results (Table I) surprisingly re eal that VO_2 during shallow rapid panting is generally less than during normal breathing at thermoneutrality Measurements were only done if the animals accepted the face mask for 4-7 min periods and if the depth and rate of breathing continued unchanged.

Expt.	T	T	Breaths/min	VO_2 (ml O_2 STPD/g/h)	VO_2 % of value at thermoneutrality
1	2[illegible]	39 0	18	0.55	100
	43	39 0	100	0.55	100
	43	39.2	210	0.58	105
2	23	38.3	70	0.57	100
	43	39 4	180	0.34	60
	43	39.8	120 230	0 46	81
	43	39.8	170 230	0.39	68
	28	38.2	30	0.50	88
3	21	38.0	4	0.54	100
	40	40 1	240	0.35	64
	40	40.3	168	0.34	63
	24	39.0	20	0.515	94
4	27	–	38	0.5	100
	37	40 4	42	0.68	130
	35	41.5	[illegible]50	0.39	75
	35	41 7	330	0.30	58

A reduced VO_2 during panting has to our knowledge not hitherto been reported. It must be associated with a reduced alveolar ventilation in spite of the much elevated total ventilation. The efficiency of thermal panting in heat dissipation of the Dik Dik is unquestionable. A prolonged duration must, however jeopardize the continued provision of O_2 for aerobic metabolism.

The behaviour pattern of the Dik Dik is suggestive that the described response may afford an emergency short term mechanism for intermittent heat dissipation which may enable this miniature antelope with a very low thermal inertia to survive brief periods of intensive heat stress

Reference

G. M. O MALOIY Proc. Roy Soc. London B *184* 167–178

C 10

Tensile Strength of Gas Saturated Biological Liquids Exposed to Successive High Pressures

By LARS OLE KORNUM LEIF BJØRNØ, PER KRAG CARL HELGE NIELSEN and POUL-ERIK PAULEV *Institute of Medical Physiology B University of Copenhagen and Department of Fluid Mechanics Technical University of Denmark*

A liquid under pressure is being saturated with a given gas—Argon Nitrogen Carbon dioxide and Air—by circulation through a column of gas exchangers. A sample of the gas saturated liquid is isolated in a test chamber the volume of which may be increased by means of a moving piston. The piston motion is cyclical with a variable frequency The pressure in the test chamber is measured by means of a capacitive pressure pick-up. When the volume increase of the gas saturated liquid in the test chamber is being compensated by

the development of gas phase bubbles the pressure decrease will stop. The recording device will show a pressure plateau or just a turn in the pressure-time course depending on the relationship between the velocity of the growth of the bubbles and depending on the piston motion. The tensile strength revealed to be independent of the frequency of the cyclical pressure decrease within the frequency limits 1 Hz down to 10^{-4} Hz. Most experiments were carried out at one single frequency of 0.5 Hz.

In the treatment of decompression sickness it has long been known that oxygen has a substantial beneficial effect in the last stages of recompression procedure. The biological mechanism implied is unknown (Elliott, Hallenbeck and Bove 1974 Royal Navy Diving Manual 1972, U.S. Navy Diving Manual 1973).

Measurements with the above method on different liquids saturated with different gases showed that biological liquids saturated by gases containing oxygen showed a remarkable higher resistance (approximately 3 times higher in pig serum) to bubble formation and a lower resistance to resolve by recompression.

Oxygen increases the surface activity of biological liquids. This effect may offer an explanation of the unknown biological mechanism stated above.

References

ELLIOTT, D. H., J. M. HALLENBECK and A. A. BOVE, *Lancet 1974 14* 1193–1199
Royal Navy Diving Manual. H. M. Stationery Office 1972.
U.S. Navy Diving Manual, Navships. Washington, D.C., 1973

C 11

Ion Transport in Non-acid Secreting Gastric Epithelium

By GUNNAR FLEMSTRÖM *Department of Physiology and Medical Biophysics University of Uppsala, Sweden and Department of Physiology and Biophysics University of Alabama in Birmingham, USA*

The gastric antral mucosa is of considerable physiological interest. This region separates the secretory epithelium of the gastric fundus from the absorptive epithelium of the small intestine and its properties are important in relation to control of gastrin release. The antral epithelium is composed mainly of surface epithelial cells while transport in the fundus is dominated by the secretion of hydrochloric acid, presumably originating from the parietal cells. The aim of the present study has been to examine ion transport in the antrum and the non-acid secreting fundus.

Well defined isolated *Necturus* antral mucosae were mounted in chambers. Flux studies showed that both sodium and chloride were actively transported sodium from the luminal to the nutrient (interstitial) side and chloride in the opposite direction. Only the sodium transport contributed to the electrogenic properties of the tissue as evidenced by abolition of the transepithelial electric potential difference on replacement of sodium with choline and the inhibitory effect of amiloride (10^{-4} M luminal side) and ouabain (10^{-4} M, nutrient side) on the electrogenic properties and net sodium flux. Microelectrode studies showed a amiloride sensitive sodium conductance in the luminal cell membrane and potassium ar

chloride conductances in the nutrient cell membrane both cell membranes being p able also to bicarbonate

Antral mucosae from *Necturus* and *Rana catesbeiana* were capable of alkaliniza the luminal side solution and this was arrested by inhibitors of tissue metabolis inhibited by acetazolamide Addition of bicarbonate to the nutrient side increased th of alkalinization and the transepithelial electric potential difference. An alkalinizat the luminal solution with the same sensitivity to inhibitors as that in the antrum was in fundic mucosae from *Rana catesbeiana* and *Rana temporaria* when acid secretio either inhibited by thiocyanate or the H_2 receptor antagonist Burimamide, or spo ously absent. The rate of alkalinization in both fundus and antrum amounted to ap mately 10 percent of maximal fundic hydrogen ion secretion *in vitro*

It is suggested that the alkalinization may protect the luminal surface of the epithelium from intraluminal acid and that this might explain previously reported (W *et al* 1965) ulcerogenic effects of acetazolamide.

References

FLEMSTRÖM G and G SACHS, *Amer J Physiol* 1975 (in press).

FLEMSTRÖM G In *Mechanisms of H secretory processes* D K KASBEKAR, ed Deccar In York, 1975 (in press)

WERTHER, J L F HOLLANDER and M ALTAMIRANO, *Amer J Physiol*. 1965 *209* 127 133

C 12

Aspects of the Central Interaction between Excitatory Chemoreceptor and Inhi Baroreceptor and Cardiac Receptor Influences

By GÖRAN WENNERGREN ROD LITTLE and BENGT ÖBERG, *Department of Phy gy University of Göteborg Sweden*

Our present knowledge of cardiovascular reflex control emanates mainly from st where individual reflexes have been separately studied while disturbing influences other receptors have been eliminated However in most situations callin cardiovascular adjustments there is usually a simultaneous engagement of several refl and the observed responses therefore constitute an integrated net effect of several I (*cf* Korner 1971) During hypoxia for instance there occurs a concomitant activati arterial chemo- and baroreceptors and also of cardiac ventricular receptors (Thorén I To analyse the vasomotor centre response to such an input profile of mixed *excit* (chemoreceptor) and *inhibitory* (baro- and ventricular receptors) influences experi were performed on anesthetized artificially ventilated cats. Baro- and chemorece reflexes were produced by perfusing the isolated carotid sinus regions at varying press with arterial or venous blood Ventricular receptor afferents in the right cardiac nerve electrically stimulated

The results suggest that a simultaneous chemo- and baroreceptor activation impl simple summation of excitatory and inhibitory influences on medullary vasom neurons while the interaction between chemoreceptor and cardiac receptor influences

...art complex. Thus, the vasodilator responses to cardiac nerve stimulation were effectively suppressed by a concomitant chemoreceptor stimulation, provided that a primary ...tor bradycardia occurred. In contrast, the bradycardia response to cardiac ...lation was essentially uninfluenced by alterations in chemoreceptor activity ...xperiments cats were exposed to severe hypoxia (ventilation with 3% O_2, 4% ... leading to a marked reflex bradycardia and vasoconstriction. Interruption of the ...hways from cardiac receptors eliminated the bradycardia response but did not ...asoconstrictor response to hypoxia. This again demonstrates that while the ...receptors may contribute significantly to the reflex bradycardia during hypoxia ...t capable to exert their reflex vasodilator influences in the presence of a strong ...ptor stimulation. This interaction between the two reflexes may be particularly ...ming diving in which situation a maintained strong vasoconstriction is urgently ...pite a proposed activation of ventricular receptors (Blix et al 1974).

References

...R. L. Gautvik and H. Refsum, Acta physiol. scand. 1974. 90 289–296.
...L, Physiol. Rev 1971 51 312–367
... Acta physiol. scand. 1972. 85 455–463.

C 13

...ynthesis and Lactate Metabolism during Recovery after Maximal Exercise in Man

...ermansen L. O Vaage, C Williams, A. Helland and L. Nilsen, Institute ...rk Physiology Oslo Norway

...actate can be resynthesized to glycogen in skeletal muscle as was suggested by ...(1920) and Hill (1932), is still an open question. Studies by Cori and Cori (1929) ...al other investigations indicated that the liver rather than the muscle, was the ...site for lactate removal during recovery after exercise Later the possibility of ...g lactate into glycogen in the muscles has been questioned (Krebs and Woodford ...e to the fact that only one of the key enzymes for a reversal of the glycolytic ...could be demonstrated

... studies by Hermansen et al (Unpublished results) showed that very little lactate ...mmol/min) escaped from the muscles during recovery after maximal exercise. ...dies (Karlsson 1971) have shown a rapid fall in the lactate concentration in the ... These studies raised the question again as to whether part of the lactate might be ...d into glycogen in the muscles.

...lthy young male subjects performed 3 times one min of maximal exercise to ...on on bicycle ergometer with a short rest period of 4 min inbetween. After the ...ercise bout, the subjects rested for 30 min. Muscle biopsies (from the lateral part of ...triceps muscle) and blood samples were taken at rest before exercise, immediately ...third exercise bout and at the 5th, 10th, 20th and 30th min of the recovery period. ...e lactate concentration increased from 0 9±0.1 mmol kg wet weight at rest, to ... 0 mmol kg wet weight after the third exercise bout. During the recovery period ... lactate concentration decreased to 5.8±0.8 mmol kg wet weight. The muscle

glycogen content decreased from 78 7±4 0 mmol kg⁻¹ wet weight (average value ±S E.), to 51 0±6 7 mmol kg⁻¹ wet weight after the third exercise bout (p<0 001) During the recovery period glycogen content increased to 60 2±4 2 mmol kg⁻¹ wet weight (p<0 001) It is concluded that the rapid synthesis of glycogen observed during the recovery period is partly explained by a resynthesis of lactate into glycogen in the muscles.

References

CORI C F and G T CORI *J Biol Chem* 1929 *81* 389–403
HERMANSEN L. O VAAGE, E. PRUETT and S MAEHLUM, *Unpublished results*
HILL, A. V *Science* 1932. *11* 345–355
KARLSSON J *Acta physiol scand* 1971 Suppl 358.
KREBS, H A and M WOODFORD *Biochem J* 1965 *94* 436–445
MEYERHOF O *Pflügers Arch ges Physiol.* 1920. *182* 284–317

C 14

Initial Effects of Endotoxin on Autonomic Efferent Discharges in Dogs

By M O HALINEN M O K. HAKUMÄKI and H S S SARAJAS. *Departments of Physiology University of Kuopio Kuopio and College of Veterinary Medicine Helsinki Finland*

From the behavior of the plasma catecholamines and heart rate it has been concluded that i v endotoxin activates the sympathetic (Lillehei *et al* 1964) and vagal systems (Chien *et al* 1966) To test the validity of this indirect evidence we have explored the initial effects of endotoxin on autonomic efferent discharges in 10 mongrel dogs premedicated with s.c. morphine hydrochloride (1 mg/kg) and anesthetized with i.v chloralose (100 mg/kg). Endotoxin *E coli* (Difco) was given i.v 1 mg/kg. In the operative period the acid-base balance was adjusted to the physiological limits Following thoracotomy and other preparative procedures, the efferent impulse activities were recorded from the cardiac postganglionic sympathetic fibers and from vagal fibers of the left cervical vago-sympathetic trunk.

After a latency of some 30 s endotoxin elicited a sharp partly reversible drop of blood pressure with an associated cardiac acceleration Concurrently the cardiac sympathetic efferentation showed within a period up to 6 min a 1.5-fold increase (p<0 001) as compared to the control level Thereafter the sympathetic discharges were largely normalized The vagal efferent discharges immediately showed a slight transient reduction (N.S) but some 2 min following the injection there was a 1 4-fold increase (p<0 001) in the vagal efferentation as compared to the control level Subsequently the vagal activity too, showed a trend to restoration

Our results are compatible with the findings of Chien *et al* (1966) who concluded from heart rate recordings and experiments with sympathectomy that endotoxin evokes vagal activation with an antecedent transient reduction of vagal activity More relevantly our results concur with the observations of Thämer *et al* (1968) In dogs and cats with hemorrhagic shock, these workers found increased cardiac sympathetic efferentation during fast reduction of arterial pressure. Thus, in turn corroborates the concept of Lillehei

) that in dogs, though not in man, endotoxin shock resembles hemorrhagic
) in physiological characteristics.

References

. CHANG, R. J. DELLENBACK, S. USAMI and M. I. GREGERSEN, *Am. J. Physiol.* 1966, 210

. C., J. K. LONGERBEAM, J. H. BLOCH and W. G. MANAX. In: Schock. Ed. S. G. Hershey
& and Company. Boston 1964. Pp. 139–205
H. WEIDNER and F. KIRCHNER. *Z. Kreislaufforschg.* 1968, 58 472–484

C 15

d Metabolic Effects of Local Cooling in Canine Subcutaneous Adipose Tissue

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us adipose tissue is presumed to function as an insulator during cold exposure. rides a major store of metabolic energy. In the present study the vascular and adaptations to lowered temperature were studied in canine subcutaneous one *in situ*.

inal fat pad preparation (Ngai *et al.* 1966) was enclosed in a chamber perfused at a controlled temperature, and its vascular resistance and release of lipolytic was studied at 37 and 26°C. Local cooling increased resting vascular resistance 49 $p<0.02$). Moreover the percentage decrease in vascular conductance induced by ed nerve stimulation (4 Hz for 5 min) was enhanced by cooling (72 per cent at 3 per cent at 37°C, $p<0.05$). The vasoconstrictor response was also prolonged latory hyperemia was considerably delayed in time and decreased in magnitude ooling.

stimulation at normal temperature glycerol release increased little. After cessa stimulation glycerol release to peak value within 1 min. 20–30 min later release to normal. At 26°C glycerol release was considerably delayed: the peak release rred 6 min after the termination of nerve stimulation and release had not com-turned to normal eve 45 min after stimulation. The total amount of glycerol was unchanged by cooling (23±6 *vs* 20±5 μmol/100 g). The time course of FFA was similarly affected by cooling but the total amount released was decreased ±8 to 22±8 μmol/100 g) ($p<0.01$).

bserved increase in resting vascular resistance as well as the potentiation of ic vasoconstriction might lead to increased thermal insulation. Lipolysis was, r affected to a minor degree enabling the tissue to provide adequate metabolic fuel r parts of the body.

Reference

H. S. ROSELL and L. WALLENBERG. Nervous regulation of blood flow in the subcutaneous se tissue in dogs. *Acta physiol. scand.* 1966, 68, 397–403.

C 16

On the Functional Significance of the so-called Pneumotaxic Centre for the Respirator Pattern Generation

By CURT VON EULER, IRJA MARTTILA, JOHN REMMERS and TERESA TRIPPENBACH
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On the basis of previous results from this laboratory it was suggested by Bradley *et a* (1975) that apneusis would occur under conditions when the threshold for the inspirator off-switch neurons is not reached by the input from the neurons in the solitary tract whe pulmonary stretch receptor activity combines with a centrally generated inspiratory activ ity (CIA) Such a condition may occur as the results of 1) an increased threshold or 2) depressed CIA The present experiments were performed to test the above hypothesis.

Cats under light pentobarbitone anaesthesia were paralyzed by gallamine and artificial ventilated 1) either proportionally to the efferent phrenic activity using a servo-respirato 2) or at a fixed rate and stroke volume Apneusis was obtained by restricted lesions with the medial parabrachial nucleus in rostral pons (the site of the pneumotaxic centre (Bertrand and Hugelin 1971)) and a broken volume feedback from the lungs

It was found that the apneustic phrenic activity always reached higher values than t peak activity at inspiratory termination in rhythmic breathing The apneustic activity of depended on PCO_2 with the same sensitivity as in rhythmic breathing (Stella 1938) and wa a normal dependence of the initial rate of rise of inspiratory activity on PCO_2 and bod temperature. In apneusis as in intact cats both the inspiratory off-switch threshold an the CIA increased together Consequently the duration of apneusis changed relatively litt with CO_2. With increased body temperature however only CIA increased (Euler an Trippenbach 1975) with the results that apneusis duration decreased and normal rhythm cal breathing was resumed even without vagal feedback. The Hering-Breuer volum threshold for inspiratory termination was increased following the rostral pontine lesion

Our results indicate that the so called pneumotaxic centre merely provides an excita ory threshold-lowering input to the inspiratory off switch mechanism without necessari influencing the characteristics of either the CIA generation or the CIA vagal interaction.

References

BERTRAND, F and A. HUGELIN J Neurophysiol 1971 34 189–207
BRADLEY G. W C. VON EULER, I MARTTILA and B ROOS, *Biol Cybernetics* 1975 In press.
EULER, C. VON and T TRIPPENBACH 1975 To be published
STELLA, G., *J Physiol.* (Lond.) 1938, 93 263–275

C 17

Isolated Gastric Glands: A New Approach for the Study of Gastric Secretion

By THOMAS BERGLINDH, *Department of Physiology and Medical Biophysics Un versity of Uppsala Sweden*

For the investigation of gastric secretion it is necessary to use both *in vivo* and *in vit* technics The most useful isolated preparation so far has been the amphibian gastr

Different methods have been applied to use isolated mammalian tissue in the same ...nsport and Jensen Chavre 1950, Kitahara *et al.* 1969) but the results ha e been ...tory Isolated parietal cells have been separated from mammalian mucosa (Croft ...finger 1969 Berglindh and Öbrink 1973) but these cells have shown little or no ...to gastric secretagogues.

...ea therefore arose to separate the mucosa into functional units, i.e. into isolated ...lands. This was made possible by high pressure perfusion of a rabbit stomach, ...l by collagenase enzyme treatment of minced pieces of the corpus mucosa.

...lands obtained in this way were highly viable and had an intracellular morphology ...d not differ from that seen in cells from intact animal in the electron microscope ...nds contained essentially parietal cells and zymogen cells.

...lands showed an oxygen consumption which was constant for at least 3 hours Due ...merous parietal cells in the gland the respiration was high 12.80±0.22 µl O_2/mg ...ght and 90 min (±S E. n=105).

...lular responses were tested for with the follow ng common gastric secretagogues. ...ine, carbachol, pentagastrin dibutyryl cyclic AMP and aminophylline Three dif ...parameters were studied a) oxygen consumption, b) degree of accumulation of the ...ase aminopyrine in the glandular compartment and c) stereological measurements of ...s in parietal cell morphology

...amine and db-cAMP effected all three parameters, whereas carbachol only in...d the respiration and pentagastrin had no stimulatory effect. If the phos...sterase inhibitor aminophylline was added to histamine stimulated glands, the ...nse was strongly potentiated.

...results suggest that histamine is the final mediator of parietal cell stimulation and ...works via the stimulation of adenyl cyclase

...s new gastric gland preparation has also bee used to study inhibitors of gastric ...tion, as well as to analyse the lectrolyte content in resting and stimulated glands.

...s concluded that isolated gastric glands provide a new useful tool by which the ...ate mechanisms of gastric acid secretion can be studied.

References

...LINDH T and K. J. ÖBRINK, *Acta physiol. scand.* 1973 *87* 21 A–22 A.
...T D N and F J INGELFINGER, *Clin Sci.* 1969 *37* 491 501
...NOR H W and V JENSE CHAVRE, *Gastroenterology* 1950 *15* 467–480.
...HARA S K R POX and C A M. HOGBEN, *Dig Dis* 1969 *14* 221–238.

C 18

Role of Parasympathetic Bronchomotor Fibres in Airway Constriction after Sudde... ...vascular Platelet Aggregation

By JARLE VAAGE *Department of Physiology University of Oslo Norway*

...monary macroembolism with platelet aggregates is a pathogenetic component in t... ...elopment of the respiratory distress syndrome of the adult (Blaisdell 1974). Experime... ...ly induced platelet aggregation in vivo has been shown to cause a reduction in dynam...

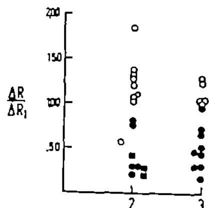


Fig. 1 Nonelastic pulmonary resistance responses to the second and third collagen infusions (Δ R) are given as fractions of the response to the first infusion (Δ R_1). The number of each individual collagen infusion is shown on the abscissa. ○ controls. ● vagotomy ■ atropinization.

lung compliance (dyn C_L) and a rise in nonelastic pulmonary resistance (R_L) as well as in pulmonary vascular resistance (PVR) partly due to the release of bioactive substances from platelets (Vaage Bø and Hognestad 1975) The role of vagal reflexes in these responses has been investigated in the present experiments

In open-chested cats during continuous positive pressure ventilation with a constant tidal volume platelet aggregation was induced by i v infusions of a solution of collagen fibrils Three repeated infusions in control animals had the effect on dyn C_L, R_L and PVR described above The three infussions gave almost identical lung responses. Bilateral cervical vagotomy carried out before the second (or the third) collagen infusion reduced the rise in nonelastic pulmonary resistance (Δ R) whereas the changes in dyn C_L and PVR were unaltered as compared to the first collagen infusion. Atropinization had the same effect as vagotomy The effects of vagotomy and atropine on Δ R are summarized in Fig. 1

Administration of indomethacin (?0 mg/kg i v) to inhibit platelet aggregation abolished the responses to collagen infusions

These observations suggest that sudden intravascular platelet aggregation induce reflex bronchoconstriction predominantly of conducting airways in which efferent parasympathetic vagal nerve fibres participate The link between platelet aggregation and reflex bronchoconstriction is unknown

References

BLAISDELL, F W., *Arch Surg* 1974 *108* 44–49
VAAGE J B Bø, and J HOGNESTAD, *Acta physiol. scand* 1975 In press.

C 19

Adrenoceptor Mediated Positive and Negative Feedback Control of Noradrenaline Secretion from Human Vasoconstrictor Nerves

By L. STJÄRNE. *Department of Physiology Karolinska Institutet Stockholm Sweden*

Isolated superfused biopsy specimens of peripheral arteries and veins, obtained from patients undergoing surgery were used for studying factors controlling the secretion of sympathetic neurotransmitter The tissues were preincubated with ^{3}H-noradrenaline (NA)

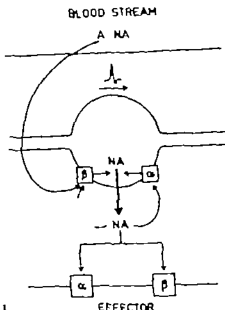


Fig. 1

...retion of NA was monitored by measurement of the secretion of ^{3}H–NA evoked ...mulation of the vasoconstrictor nerves, with trains of 300 shocks at 1 Hz.

...us NA 0.2–1 μM dose-dependently and reversibly depressed the secretion of ...d α-adrenoceptor blocking drugs enhanced ^{3}H-NA secretion. This indicates that ...soconstrictor nerves possess an α-adrenoceptor function capable of sensing the ...tal concentration of their own transmitter and of triggering restriction of further ... of such transmitter. These receptors appear to be set at a sensitivity level ...ding to the high NA concentrations in the synaptic cleft, they are too insensitive ... NA at the concentrations in which it normally occurs in the general circulation. ... seems likely that human vasoconstrictor nerves possess an α-adrenoceptor ...m mediating autoinhibition of NA secretion (Fig. 1).

...cretion of ^{3}H-NA appears in addition to be controlled by a β-adrenoceptor ...unc the β agonist isoprenaline already at a concentration of 1.6 nM signific...anced the secretion of ^{3}H NA. At 40 nM the increase was 67.7±6.2 %. However ...cking drug propranolol, 0.1–1 μM did not significantly depress ^{3}H NA secretion. ...appears that the secretion of sympathetic neurotransmitter in these nerves is not ... dependent on a β-adrenoceptor mediated positive feed-back loop. On the other ...renaline significantly enhanced the secretion of ^{3}H-NA, already at a concentration ... M. The effect was "biphasic" at concentrations higher than 40 nM adrenaline ...ed ^{3}H NA secretion even more strongly than did NA.

...results show that the secretion of ^{3}H-NA from human vasoconstrictor nerves is ...ed by adrenaline at concentrations normally occurring as a result of stimulation of ...medullary secretion, and depressed by NA at concentrations normally occurring in ...naptic cleft (Fig. 1).

C 20

Hormone Stimulated K⁺ Transport in Isolated Rat Liver Cells

By TROND BERG and JENS-GUSTAV IVERSEN *Institute of Medical Biology Univ of Tromsø Norway*

The effects on target cells of a large group of hormones are dependent on an initial b of the hormone to a specific receptor on the cell surface Very soon after this interact increase in membrane transport of *e g* ions can be observed (reviewed by Riggs 197(want in this study to characterize more precisely the hormone induced changes i transport.

Unidirectional K^+ fluxes were therefore measured in suspensions of isolated rat parenchymal cells (Berg *et al* 1972) incubated with ^{42}K in vitro By tracer exct analysis fluxes in both directions were estimated to 8–9 10^{-12} mol/cm² sec

Glucagon in concentrations above 2 10 ⁸ M increased both influx and efflux to 160 control values. Insulin (100 μU/ml) increased influx by 12–14%, whereas efflux apparently unaffected

Using an extracellular marker ⁵ Cr EDTA the intracellular level of some ions estimated in the isolated liver cells K =172 mmol/kg water Na =25 mmol/kg w Cl=53 mmol/kg water Cellular water content: 60%

Incubation with insulin for 1 h increased on an average intracellular concentration of 1 7 mmol/kg water The results indicate that glucagon increases primarily the permeability of the cell membrane while insulin stimulates active K transport into cell

References

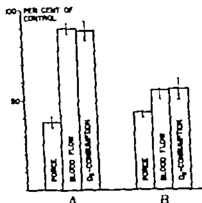
BERG, T D BOMAN and P O SEGLEN *Exp Cell R s* 1972, 72 571–574
RIGGS, T R. in *Biochemical Actions of Hormon s* Vol I Ed G Litwack, Academic Press, N York 1970 157–208

C 21

Reduced Muscle Efficiency during Vibration-induced Attenuation of Contractile Force in C Soleus Muscle

By B LJUNG R. SIVERTSSON M HALLBACK and B FOLKOW *Department of Physiology University of Göteborg Sweden*

Vibrational changes in muscle length have been shown to reduce the isometric contractil force in both skeletal muscle (*e.g* Joyce Rack and Westbury 1969) and smooth muscl (Ljung and Sivertsson 1972). It was suggested in these reports that vibration might interfer with the contractile mechanism by causing an increased detachment of the actin-myosin cross-links In order to further elucidate the nature of the relaxing effect exerted by longitudinal vibration isometric force regional blood flow and oxygen consumption were measured in the acutely denervated soleus muscle preparation of the cat (Folkow and Halicka 1968) In the control situation the steady state values for active force blood flow and O_2 consumption were measured during repeated single twitch activations of the soleus



. 1. Changes in active force, regional blood flow and gen consumption in cat soleus muscle from control ations—where repeated single twitch activations (4 , 0.2 ms, 4 V) are set as 100%—to the situations il- rated in A caused by longitudinal vibrations (50 ±0.5 mm) and B caused by reduction of the number of vated motor units by lowering nerve stimulation in- ity from 4 V to 0.4 V. Mean±S.E., =10.

ncle by supramaximal nerve stimulation at 4 Hz, and set at 100% Vibrations at 50 Hz th an amplitude of ±0.5 mm were applied to the muscle tendon via the force transducer d led to an immediate decrease in twitch force. On the average the contraction amplitude s reduced by 60% but blood flow and O_2 consumption were only slightly reduced from e control alues (Fig. 1 A). In contrast when a corresponding decrease of acti e muscle rce as obtained by reducing the number of activated motor units (as a result of lowered mulation intensity) a considerable reduction in blood flow and O_2 consumption ensued ig. 1 B).

It is concluded that longitudinal vibrations interfere with the contractile process after the ergy needed for the generation of force has already been expended. The results thus pport the hypothesis that the mechanical movements increase the rate of cross-link tachment, which would seem compatible with the finding of essentially unchanged ergy consumption during vibration-induced attenuation of contractile activity.

References

…ILL, G. C. P. M. RACK and D. R. WESTBURY, *J. Physiol.* (Lond.). 1969. 204. 461–474.
…KOW, B. and H. D. HALICKA, *Microvascular research* 1968. 1. 1–14.
…NG, B. and R. SIVERTSSON, *Acta physiol. scand.* 1972. 85. 428–430.

C 22

…laminar Distribution of U-fibres from the Postcruciate Cerebral Projection Area of the …at's Group I Muscle Afferents

By G. GRANT, S. LANDGREN and H. SILFVENIUS. *Department of Physiology, University of Umeå, and Department of Anatomy, Karolinska Institutet, Stockholm, Sweden*

Group I muscle afferents from the contralateral forelimb project to area 3a near the postcruciate dimple, Pcd (Oscarsson and Rosén 1963, 1966) and to area 4γ near the lateral end of the cruciate sulcus (Silfvenius 1972) of the cat's cerebral cortex. However Phillips, Powell and Wiesendanger (1971) did not find any responses to group I muscle afferents in the baboon's area 4γ. The cortical potential evoked in area 4γ of the cat, was of comparatively low amplitude and generally showed latency which was 1–2 ms longer

than that of the potential evoked in Pcd The question was therefore raised whether th Group I response evoked in 4 γ was relayed via area 3 a.

In the present series of experiments needle stitch lesions restricted to cortex cerebri an with a maximal diameter not exceeding 500 μm were placed in the point near Pcd whe the response to electrical stimulation of the low threshold afferents of the contralater deep radial nerve was of maximum amplitude The degeneration of nerve terminals wa studied with Fink Heimer technique after survival times of 26 48 and 96 h.

Terminal degeneration was observed in area 4 γ near the lateral end of the crucia sulcus as well as more medially near the location of the projection area of the Group muscle afferents of the contralateral hind limb (Landgren and Silfvenius 1969) Termin degeneration was also found in area 2 in the rostral bank of the lateral ansate sulcus, and area 3 b near the caudal end of the coronal sulcus Terminal degeneration was observed the forelimb area of SII in some of the animals

The connection between the lesion and the degeneration areas was found to be U-fibr travelling in the white matter and reentering the cortex in a radial direction Abundant th arborizations were observed in layer III and typical terminal degeneration in layer I of th cortex The degenerations were restricted to distinct columns with a diameter of abo 1 000 μm. Up to 4 clearly separated columns were observed in area 4 γ

The observed connections between the projection areas of the low threshold musc afferents in area 3 a and 4 γ may subserve the group I path to the motor cortex.

References

LANDGREN, S and H SILFVENIUS, J Physiol 1969 *200* 353–372.
OSCARSSON, O and I ROSEN J Physiol 1963 *169* 924–945
OSCARSSON, O and I ROSEN J Physiol 1966. *182* 164–185
PHILLIPS, C. G T P S POWELL and M WIESENDANGER, J Physiol. 1971 *217* 419–446.
SILFVENIUS, H Umeå University medical dissertations. 1972 No. 4

C 23

Water Permeability in the Human Forearm during Graded Exercise

By T PALM S L. NIELSEN and N A LASSEN *Department of Clinical Physiolog Bispebjerg Hospital Copenhagen Denmark*

It is difficult to measure changes in water permeability in skeletal muscle during rhythm exercise with plethysmographic techniques but in animal experiments an increase i capillary filtration coefficient (CFC) with work load has been observed Using an osmoti transient technique it is possible in the human forearm to study the water permeabili without changing the prevailing hydrostatic and crystalloid osmotic pressures. The osmo tic gradient is created by close intraarterial infusion of 25 % human albumin solutio dialyzed to crystalloid osmotic isotonicity and the water concentration in the bloo samples from the deep veins of the forearm can be determined from concentration of water marker in the arterial blood ([131] Iodide-albumin) and infused solution ([131]Iodide albumin) The principle has recently been used in dog lungs with crystalloids as osmoti driving force (Effros 1974) In the present *study on normal subjects* graded exercise wa

c performed with a hand ergometer and blood flow was measured by dye-dilution technique and strain gauge plethysmography

...est the amount of water that was sucked into the blood during a *single passage* ...h the vascular bed in the muscles showed a linear relation to the suction forces i.e. ...otic pressure of albumin. Knowing the relative blood flow a capillary filtration ...ient can be calculated from the amount of water per ml blood per mm Hg times flow ...FC values were somewhat higher than found by measurements with plethysmograp-...chnique. With increasing work load the *amount of water* sucked into each ml of ...*ing blood was independent of blood flow* This means that a linear relation existed ...en CFC and blood flow in the range from resting values to maximal dilatation. If it is ...ed that water permeability does not change during exercise then changes in CFC ...tes changes in surface area, i.e. surface area increased in proportion to the *change in* ...n consonance with *the concept formulated by* Krogh or recruitment of capillaries ...ncreasing work load

References

...n, R. M., *J. Clin. Invest.* 1974 *54* 935–947

C 24

...ronmental Adaptation and Multiplicity in Hemoglobins of the Flatfish Plaice and Floun-

By ROY E. WEBER, *Department of Zoophysiology University of Aarhus Denmark*

...e (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) are inactive flatfish, in-...ing coastal benthic habitats. Flounders, however occur closer inshore than plaice and ...thus subject to greater variation in ambient conditions. Flounders accordingly show ...ter tolerance to environmental temperature and salinity than plaice (Waede 1954). This ...r reports the oxygenational properties of the hemoglobin systems of these fish, ...mpting to trace the possible adaptations to environmental conditions.

...he hemoglobin concentration in flounder blood *is about 50 per cent higher than in* ...ce. Flounder hemoglobin has a higher oxygen affinity than plaice in the red cells, as ...l as in purified solutions. Flounder hemoglobin is moreover less sensitive to pH, both as ...ards oxygen affinity (Bohr effect) and oxygen capacity (Root effect).

...ompared to many other fish, *the ratio of ATP (a potent regulator of oxygen affinity in* ...t blood) to hemoglobin is low in both species, amounting to about 1.5 The ...P-hemoglobin ratio is however well adjusted to the fact that ATP exerts greater ...uence on P_{50} (half-saturation oxygen tension) of plaice hemoglobin at low concentra-...n ratios (Weber and De Wilde 1975).

...In both plaice and flounder the main hemoglobin components (separated by iso-electric ...using) show similar Bohr and ATP effects, and these properties moreover correspond to ...se of the whole hemolysates (Figure 1) lacking evidence for interaction between ...occurring components *in vivo* In their functional similarity the *multiple* hemoglobins ...plaice and flounder differ from those in trout, salmon and eel, which contain pH

than that of the potential evoked in Ped The question was therefore raised whe Group I response evoked in 4 γ was relayed via area 3 a.

In the present series of experiments needle stitch lesions restricted to cortex ce with a maximal diameter not exceeding 500 μm were placed in the point near Pc the response to electrical stimulation of the low threshold afferents of the cont deep radial nerve was of maximum amplitude. The degeneration of nerve termi studied with Fink Heimer technique after survival times of 26, 48 and 96 h.

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References

LANDGREN S. and H. SILFVENIUS J Physiol 1969 200 353–372.
OSCARSSON, O and I ROSEN J Physiol 1963 169 924–945
OSCARSSON, O and I ROSEN J Physiol 1966. 182 164–185
PHILLIPS C G T P S POWELL and M. WIESENDANGER. J Physiol. 1971 217 419–446.
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C 23

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ADIPOSE TISSUE

...elative change in total blood flow ... (R_{test}/R_{ref}) is plotted against ...rnal blood pressure ($\bar{P}_a$). The open ... nd the dotted line refer to experi-...erformed on the distal part of ..., the filled circles and the unbroken ... experiments on the distal part of ... he p-values indicate significance ...ween responses obtained in forearm ... as calculated by the randomization ... two independent samples. The ...tal bars signify 1 S.E.

... to an increase in transmural pressure was more pronounced in orthostatic ...ensive crus than in normotensive forearm (Fig. 1).

...evels above the heart where venous pressure is constant autoregulation of blood flow ...resent in a wider arterial blood pressure range in crus than in forearm. When ...ural pressure increased 25 mm Hg or more in the dependent position vascular ...ance increased approximately 50 per cent in forearm and 300 per cent in crus.

... influence of induced ischemia on vascular resistance was investigated in cutaneous ... of hand and foot by means of the [131]I Antipyrine initial slope technique. Vascular ...ance after maximum dilation achieved after 20, 30 and 35 min of ischemia was less in ...s of the hand than in vessels of the foot. It is concluded that adaptive functional ...ts of vessels subjected to increased blood pressure can be demonstrated in normal ...cts.

C 26

...ts of Antidiuretic Hormone on the Intrarenal Distribution of Red Cells and Plasma in ...at

By S. Nybo Rasmussen, *Institute of Medical Physiology B, University of Copenhagen, Denmark*

...not totally clarified to what extent antidiuretic hormone (ADH) affects renal vascular ...tance. Transition from water diuresis to antidiuresis is believed to be accompanied by ...crease in renal blood flow especially in the medulla (Thurau *et al.* 1960). The purpose

Fig. 1 Half-saturation oxygen tensions (P_{50}) and their pH and ATP dependence of the main ... nents of hemoglobins from plaice (left) and flounder (right) measured at 15°C in 0.1 M T... buffers. Roman numerals represent the main components separated by iso-electric focusing ... of decreasing iso-electric point. Open symbols refer to hemoglobins that had been strip... dissolved ions closed circles refer to hemoglobins in the presence of excess added ATP (about ... excess over hemoglobin) The dashed curves represent the stripped whole hemolysates.

insensitive hemoglobin components which apparently form adaptations to tempor... creases in blood pH in more active fish

The differences in hemoglobin concentration in oxygen affinity and in pH ... indicate that flounder hemoglobin is better adapted than plaice hemoglobin for funct... under hypoxic and hypercarbic conditions in correspondence with differences ... distribution of the fish and their tolerances to environmental conditions.

References

WAEDE, M. Kieler Meeresforschungen 1954, 10 58–67

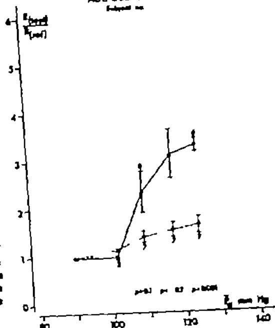
WEBER, R. E. and J. A. M. DE WILDE, J comp Physiol. 1975 In press.

C 25

Vascular Resistance in Peripheral Blood Vessels at Normotension and at Local Orth... Hypertension in Healthy Humans

By WILLIAM P PAASKE and OLE HENRIKSEN Institute of Medical Physiolo... University of Copenhagen Denmark

The influence of orthostatic pressure changes on vascular resistance in subcutan... adipose tissue was examined at normotension in the forearm and at local ortho... hypertension in the crus in 5 healthy humans Blood flow in subcutaneous tissue ... measured by means of the local xenon 133 washout technique The vasoconstricto...



..tion change in total blood flow (R_{test}/R_{ref}) is plotted against ...nal blood pressure ($\bar{P}_a$). The open ... the dotted line refer to experi... performed on the distal part of ... the filled circles and the unbroken ... experiments on the distal part of ... p-values indicate significance ... responses obtained in forearm ... calculated by the randomization ... two independent samples. The ... bars signify 1 S.E.

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C 26

...ects of Antidiuretic Hormone on the Intrarenal Distribution of Red Cells and Plasma in ... Rat

By S. NYBO RASMUSSEN *Institute of Medical Physiology B University of Copenhagen, Denmark*

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of this study was to evaluate the effects of ADH administration to water diuretic rat intrarenal red cell volumes plasma volumes and haematocrits

Water diuresis was induced in male Wistar rats by combining water loading with ethanol-thiopental anesthesia. Infusion of arginine vasopressin 1 μU/min gram ra eight rats provoked within 15 to 30 min a rise in urine osmolality from below 200 to va ranging from 800 to 2000 mOsm per kg without significantly influencing arterial bl pressure. Another eight rats served as water diuretic controls [51]Cr labelled red cells [125]I immunoglobulin M (IgM Mol Wt. = 920000) were used as tracers. 60 s elapsed fr i v injection of the tracers until clamping and freezing of the kidney The intrare distribution volumes of labelled red cells and IgM in whole kidney cortex outer and in medulla were calculated The 60 s IgM distribution volume can be taken to represent plasma volume (Rasmussen 1975)

Red cell volume was significantly increased in all renal zones following ADH administ tion whereas significant changes in regional plasma volumes could not be detected T intravascular volume (red cell+IgM volume) was higher in cortex (and whole kidne during ADH administration than in the water diuretic control situation. In all renal zon ADH caused substantial increments in haematocrit (red cell volume/intravascular volume The changes in haematocrit indicate lowered blood flow in all renal zones following ADI administration. Reduction in blood flow involves decreased linear velocities of red cel with less pronounced axial accumulation of red cells and consequently higher dynam haematocrits The reduction in local blood flow can not be explained by changes in arteri blood pressure but must be due to increased vascular resistance. Thus this study indicate that ADH increases total vascular resistance in the rat kidney The data obtained do no allow any conclusions to be drawn as regards the vascular site(s) of action of the hormone.

References

RASMUSSEN, S NYBO, *Pflügers Arch* 1975 In press.
THURAU K P DEETJEN and K. KRAMER, *Pfl gers Arch* 1960. *270* 270–285

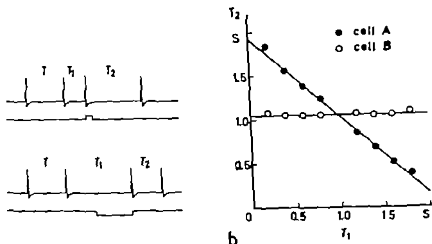
C 27

Two Types of Adaptation in Snail Neurones

By M COLDING-JØRGENSEN *Institute of Medical Physiology Dept A University of Copenhagen Denmark*

It is generally believed that the adaptation of many excitable membranes is due to an increasing hyperpolarising current but it is not clear whether the current is accumulated from spike to spike (impulse dependent) or is generated independently of spike formation (stimulus dependent) Two types of adaptation are therefore possible and can be sepa rated in the following way·

Consider a membrane firing with a constant period T The firing pattern is now inter rupted by a stimulus as outlined in fig. 1a. With impulse dependent adaptation the interposed spike will increase the hyperpolarising current, prolonging the succeeding firing period while the retardation of a spike will decrease the current, thus reducing the period.



. (a) Outline of the experimental procedure. In the upper trace spike is elicited after mean spike at a time T smaller than the spontaneous firing period T In the lower trace T is greater than T by hyperpolarising stimulus. The succeeding firing period is T in both cases. relation between the firing periods T and T for two different cells. The spontaneous firing was in both cases between 1.0 and 1 1 s.

ch spike increases the hyperpolarising current by the same amount, and the current ases monoexponentially with the time constant the relation between the disturbed he succeeding period will be almost linear for $T/\tau<0.25$ with a slope of $-1/(1+T/\tau)$ stimulus dependent adaptation, however an interruption will not influence the eding period

tion potentials were recorded by a single microelectrode from nerve cells in the ted ventral ganglion of *H lix pomatia* The firing period of the cells was kept between nd 1 1 s by adjusting a bias current through the electrode. For each value of T T was lated as an average of 5–15 samples. It appears from fig 1 b that for cell A the relation een T and *T is linear with a slope of 0.83 corresponding to a of 8 s. For cell B T* stant and equal to the mean firing period of 1.05 s

hus, two types of adaptation can be found in snail neurones, one which appears ulse dependent, and another which does not.

C 28

sient "Hypertrophy" of the Anterior Tibial Muscle after Denervation Combined with tch

By I M Krogh *Institute of Neurophysiology University of Copenhagen Denmark*

sient hypertrophy of denervated skeletal muscle of rats has been described by Brauna (1960). Already after 4 hrs the non-collagen protein content of the anterior tibial uscle was *increased by 10%* Denervation hypertrophy was observed also in the hemi-

diaphragm of rats one week after unilateral phrenicotomy and attributed to stretch by innervated hemi-diaphragm Dry and wet weight were found either increased to nearly same degree (Sola and Martin 1953 Stewart and Martin 1956 Gutmann *et al* 1966) or gain in weight was exclusively due to water (Thomson *et al* 1951 Thomson 1955). diameter of denervated muscle fibres was increased by 10% (Gutmann *et al.* 1966)

24 h after denervation of anterior tibial muscle of young rats immobilized in a stretc position Kamieniecka (personal communication) found a 10–14% increase in mean f diameter The aim of the study presented here was to investigate whether denervation-stretch hypertrophy was due to protein increase as after denervation al (Gutmann 1960) In 34 rats six weeks old the right sciatic nerve was cut and the ankle j fixed in plantar-flexion with plaster of Paris. After 24 h the animals were sacrificed. I muscles served as controls

In sections of Epon-embedded specimens mean fibre diameter was increased by 5–1 Wet weight was increased (+8 8%) Dry weight was increased (+3.5%) when the mus were dried as is customary to constant weight at 95°C (at least 24 h) However w freeze-dried under high vacuum to a final pressure of $<10^{-4}$mm Hg this differe disappeared The total amount of amino acids after acid hydrolysis was the same (−2. p=0 4) The proportion of hydroxyproline was unchanged indicating that the ratio lagen to non-collagen protein was constant The glycogen content was decreased (−5 less than 1% of dry weight) The lipid content was unchanged

This study shows that hypertrophy of muscle fibres caused by denervation and cons stretch was due to increased content of water alone

References

Gutmann E. *Physiol Bohemoslov* 1960 *9* 382–389

Gutmann, E. Hanikova, M. Hajek, L. Klicpera, M Syrovy I *Physiol. Bohemoslov* 1966 508–523

Sola, O M Martin, A. W *Amer J Physiol.* 1953 *172* 324–332.

Stewart D M Martin, A W *Amer J Physiol.* 1956. *186* 497–500.

Thomson, J D et al. *Amer J Physiol* 1951 *167* 832.

Thomson, J D., *Amer J Physiol* 1955 *180* 202–204

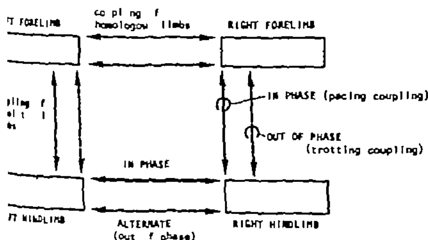
DEMONSTRATIONS

D 1

rdination of the Limbs during Locomotion in the Cat: Basic "Programs" of Movement

By J. VAN DER BURG, J. M. HALBERTSMA, F. G. A. VAN DER MECHE and S. MILLER. *Department of Anatomy, Erasmus University Rotterdam, Rotterdam, The Netherlands*

n observations in cats of movements of the 4 limbs we have concluded that the rent forms of alternate locomotion, e.g. walking, trotting and swimming, and in phase motion, e.g. galloping and jumping (Miller and van der Burg 1973) result from interac of basic programs for the phasing of flexion and extension movements of 1) the ologous limbs (hindlimbs or forelimbs) and 2) the homolateral limbs (hind- and limb of the same side). See Fig. 1A.



PLING		ALTERNATE LOCOMOTION: pace	ALTERNATE LOCOMOTION: t t swimming	IN PHASE LOCOMOTION: t gall / l rot gall / half bound	IN PHASE LOCOMOTION: jump / f ll bound
ologou	for limb				
	h ndlimb				
ol t l	left				
	ight				

1 (A) Diagram of the coupling of flexion and extension movements of the limbs. (B) Distribution of types of coupling in different gaits. tr gall right transverse gallop; l. rot. gall left rotatory lop.

In alternate locomotion flexion and extension movements of the homologous limbs c out of phase The homolateral limbs on both sides are coupled either approximate phase as in pacing or out of phase as in trotting and swimming. During in phase locom the flexion and extension movements of the hindlimbs and to a lesser extent those o forelimbs occur *approximately* in phase In the transverse rotary and half bound gal and occasionally in jumping the coupling of the movements of the homolateral liml asymmetric on one side they are coupled in phase (pacing coupling) and on the other out of phase (trotting coupling) In the full bound and usually in jumping the homolat limbs on both sides are coupled out of phase (The different types of locomotor pattern illustrated in a film) Electromyograms from the different limbs show that transit between the different types of coupling occur abruptly

The hypothesis is advanced that all the characteristic patterns of locomotion in the result from different combinations of the "programs" of homologous and homolateral li coupling (Fig 1B) Since these "programs" of coupling are probably organized ma within the spinal cord this hypothesis carries important specifications for the functio organization of long spinal and crossed segmental connections and for the supraspi control of locomotion

Reference

MILLER, S and J VAN DER BURG *The function of long propriospinal path ways in the coordination quadrupedal stepping in the cat In. The Control of Posture and Locomotion* Eds Stein, R. I Pearson, K. G Smith R. S and Redford J B Plenum Press, New York 1973 561 578

D 2

Applications of a Process Computer (WANG 600) in Experimental Physiology

By WILLIAM P PAASKE and PER SEJRSEN *Institute of Medical Physiology B University of Copenhagen Denmark*

The equipment presently available for electronic data processing at the Institute is two process computers (WANG 600) each connected to an extended memory (WANG 618) allowing an additional 4096 program steps/512 storage registers The input media are two high-speed electronic tape readers (FACIT 4022) and the keyboards. The output media are three tape punchers (FACIT 4070) and one plotter/printer (WANG 602) Two separate interface units (constructed by the Institute) can be connected to up to four parallel gamma spectrometers allowing on-line and/or tape-punch handling of data. A separate keyboard is connected to the interface units These facilities are currently being used by our group in four major research projects each to be demonstrated.

1) Determination of capillary extraction in skeletal muscle and adipose tissue by the single injection, external counting method (Sejrsen 1970 Paaske and Nielsen 1973).

2) Blood flow determination from xenon-133 washout from cutaneous, subcutaneous and muscular tissues (Sejrsen 1971 Henriksen, Nielsen and Paaske 1973 Paaske Hovind and Sejrsen 1973)

lculation of the permeability-surface area product (P_dS) and mean body capillary sion of molecules in the entire organism
etermination of diffusion coefficient in agar-agar and interstitial space of tissue.

References

...EN O S. L. NIELSEN and W P PAASKE, *Acta physiol. scand* 1973 89 531 537
...W P and S. L. NIELSEN, *Acta physiol. scand* 1973 Suppl. 396. 81
...W P H. HOVIND and PER SAMSEN, *Scand J ... Lab. Inv.* 1973 31 389–394
...P In. *Capillary Permeability* Alfred Benzon Symp. II C. Crone and N A. Lassen, eds. ...gaard, Copenhagen 1970. 256–260
...P *Measurement of Cutaneous Blood Flow by Freely Diffusible Radioactive Isotopes* ... Copenhagen 1971 Danish Medical Bulletin. 1971 Suppl. 18.

D 3

...ge Kinetics of Extravascular Albumin in the Rat Kidney Studied by Means of a Step Tracer Technique

...y S. NYBO RASMUSSEN. *Institute of Medical Physiology B University of Copenhagen, Denmark*

...g recent years the renal interstitium has received growing attention because of the ...ble significance of peritubular physical factors (*i.e* hydrostatic and oncotic pressures) ...erning tubular fluid reabsorption (Deen *et al* 1973 Pinter *et al.* 1974). The interstitial ... of plasma-proteins is in a state of turnover as there is a continuous flux of protein ...gh the pool Proteins enter from the capillaries and leave through the lymph and ...bly by way of return into the capillary blood. In order to measure the rate of ...ration of different plasma proteins into the renal interstitium a technique has been ...loped which permits to follow in time the wash-in or wash-out of radioiodinated tracer ...ns in the kidney

...two rats the caudal aortae are connected to each other by means of silicone rubber ...g; venae cavae are connected similarly The two connecting tubes are anastomosed ...an adjustable shunt placed close to the "experimental" rat, of which the left kidney is ...r study This kidney may be either autoperfused or alloperfused from the heparinized ...mp rat. A ystem of electrically operated ...ead clamps allows shifting the perfusion ...d momentarily Before starting a "cross perfusion the blood of either the "experimental" or the "pump" animal is equilibrated with radioiodinated protein, *e g* albumin. ...ing from auto- to alloperfusion or vice versa, the wash-in or wash-out of tracer in the ...ey is followed continuously by external counting The use of step input of tracer allows ...inguishing between rapid intravascular and lower extravascular components in the ...ings. The constancy of renal vascular ...olume during the experiment may be checked ...ing ^{51}Cr labelled red cells and ^{125}I labelled protein simultaneously The dead space of ...s system is extremely small and recirculation problems are minimal as opposed to the ...s injection technique

Till now only preliminary results have been obtained but the experimental set-up has

In alternate locomotion flexion and extension movements of the homologous limb out of phase The homolateral limbs on both sides are coupled either approxima phase as in pacing or out of phase as in trotting and swimming. During in phase loco the flexion and extension movements of the hindlimbs and to a lesser extent those forelimbs occur approximately in phase. In the transverse rotary and half bound, and occasionally in jumping the coupling of the movements of the homolateral li asymmetric on one side they are coupled in phase (pacing coupling) and on the oth out of phase (trotting coupling) In the full bound and usually in jumping the hom limbs on both sides are coupled out of phase (The different types of locomotor patt illustrated in a film) Electromyograms from the different limbs show that tran between the different types of coupling occur abruptly

The hypothesis is advanced that all the characteristic patterns of locomotion in result from different combinations of the "programs" of homologous and homolater coupling (Fig 1B) Since these "programs" of coupling are probably organized within the spinal cord this hypothesis carries important specifications for the fun *organization of long spinal and crossed segmental connections and for the supr control of locomotion*

Reference

Miller, S and J van der Burg *The function of long propriospinal pathways in the coordin quadrupedal stepping in the cat In. The Control of Posture and Locomotion* Eds Stein Pearson, K. G Smith R. S and Redford J B Plenum Press, New York 1973 561–578

D 2

Applications of a Process Computer (WANG 600) in Experimental Physiology

By William P Paaske and Per Sejrsen *Institute of Medical Physiology B versity of Copenhagen Denmark*

The equipment presently available for electronic data processing at the Institute i process computers (WANG 600) each connected to an extended memory (WANG allowing an additional 4096 program steps/512 storage registers. The input media a high-speed electronic tape readers (FACIT 4022) and the keyboards. The output me three tape-punchers (FACIT 4070) and one plotter/printer (WANG 602) Two se interface units (constructed by the Institute) can be connected to up to four parallel g spectrometers allowing on-line and/or tape-punch handling of data. A separate keybo connected to the interface units These facilities are currently being used by our gr four major research projects each to be demonstrated

1) Determination of capillary extraction in skeletal muscle and adipose tissue b single injection, external counting method (Sejrsen 1970 Paaske and Nielsen 1973).

2) Blood flow determination from xenon-133 washout from cutaneous subcutan and muscular tissues (Sejrsen 1971 Henriksen, Nielsen and Paaske 1973 Paaske, H and Sejrsen 1973).

lculation of the permeability-surface area product (P_sS) and mean body capillary
on of molecules in the entire organism.
termination of diffusion coefficient in agar-agar and interstitial space of tissue.

References

en, O., S. L. Nielsen and W. P. Paaske, Acta physiol. scand. 1973. 89. 531–537
W. P. and S. L. Nielsen, Act. physiol. scand. 1973. Suppl. 396. 81
W. P., H. Hovind and Per Sejrsen, Scand. J. clin. Lab. Inv. 31 1973. 31. 389–394
, P. In. Capillary Permeability. Alfred Benzon Symp. II. C. Crone and N. A. Lassen, eds.
gaard, Copenhagen 1970. 256–260
, P. Measurement of Cutaneous Blood Flow by Freely Diffusible Radioactive Isotopes
. Copenhagen 1971. Danish Medical Bulletin. 1971. Suppl. 18.

D 3

g Kinetics of Extravascular Albumin in the Rat Kidney Studied by Means of a Step 'racer Technique

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recent years the renal interstitium has received growing attention because of the le significance of peritubular physical factors (i.e. hydrostatic and oncotic pressures) erning tubular fluid reabsorption (Deen et al. 1973, Pinter et al. 1974). The interstitial f plasma-proteins is in a state of turnover, as there is a continuous flux of protein h the pool. Proteins enter from the capillaries and leave through the lymph and ly by way of return into the capillary blood. In order to measure the rate of ation of different plasma proteins into the renal interstitium a technique has been ped which permits to follow in time the wash-in or wash-out of radioiodinated tracer n in the kidney.

wo rats the caudal aortae are connected to each other by means of silicone rubber E venae cavae are connected similarly. The two connecting tubes are anastomosed adjustable shunt placed close to the "experimental" rat, of which the left kidney is study. This kidney may be either autoperfused or alloperfused from the heparinized np rat. A system of electrically operated vessel clamps allows shifting the perfusion momentarily. Before starting a "cross perfusion", the blood of either the "experimental" or the "pump" animal is equilibrated with radioiodinated protein, e.g. albumin. ng from auto- to alloperfusion or vice versa, the wash-in or wash-out of tracer in the ry is followed continuously by external counting. The use of step input of tracer allows nguishing between rapid intravascular and slower extravascular components in the ngs. The constancy of renal vascular volume during the experiment may be checked sing ^{51}Cr labelled red cells and ^{131}I labelled protein simultaneously. The dead space of system is extremely small and recirculation problems are minimal as opposed to the s injection technique.

il now only preliminary results have been obtained, but the experimental set-up has

proven to work as intended and seems to offer a powerful tool in the study of r
circulation and fluid dynamics

References

DEEN W M C R ROBERTSON and B M BRENNER *Circulation Res* 1973 *33* 1-8
PINTER, G G J L. ATKINS and D R. BELL, *Experientia* 1974 *30* 1045

D 4

Estrous Cycle of Zinc-Deficient Female Rats

By R HARTOMA. *Department of Physiology University of Oulu Finland*

Zinc seems to be necessary for the normal pregnancy and parturition of the rat. Diminis
pituitary glands and anestrous cycles have been described in severely Zn-deficient rat

In the present investigation estrous cycles of female rats fed a diet of 12 ppm of zinc w
studied

Adult female Sprague Dawley rats were used The diet was prepared by the labora
of Hässle using the normal food given to the control animals The food was available
libitum Deionised water was used Contamination through different sources was eli
nated Special cages were prepared

The follow-up of vaginal smears began after 6–7 weeks of diet. During the next 4 we
there was some increase in the weight of the animals of both groups The appearance
behaviour of the Zn-deficient rats seemed to be normal one of the animals showed hair
areas after 10 weeks of diet.

The control rats followed simultaneously had regular 4-day cycles The deficient r
had a lengthened estrus The duration increased continuously up to 7 days One of
deficient rats had after a period of a lengthened estrus a continuous anestrus with signs
an infection. After a diet of 10 weeks normal food and water was given to this animal an
was replaced in a normal cage Vaginal smears were controlled further on. The leucocy
soon started to decrease in the smears and disappeared completely in 7 days and a pict
of proestrous followed by an estrus could be observed

A lengthened influence of estrogen could be observed in the vaginal smears of Z
deficient rats. The primary change has to be detected and the level of estrogens a
gonadotropins in blood should be studied

References

APGAR, J *J Nutr* 1973 *103* 973–981
FOLLIS, R. H Jr H G DAY and E. V MCCOLLUM, *J Nutr* 1941 *22* 223–237

D 5

Effect of Local Venous Stasis on Blood Flow in Human Subcutaneous Tissue

By O HENRIKSEN *Department of Nuclear Medicine NU-2033 Rigshospitale*
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Effect of local venous stasis on blood flow in subcutaneous tissue was investigate
^{133}Xenon was applicated in the midline on the dorsal side of a forearm in an area 3 cm lo

a side half way between elbow and wrist. A lead shield (14 cm long, 0.3 cm thick 5 cm high) was placed longitudinally on the skin in the midline of the forearm ng the radioactive depot into two parts. Venous stasis was produced on one side of d by another lead shield placed on the skin proximally and laterally to the depot. low in subcutaneous tissue was measured simultaneously on both sides of the shield ns of the ^{133}Xenon washout technique.

ng venous stasis on one side blood flow decreased about 40 per cent on both sides hield. When the tissue was infiltrated with phentolamine on the side of stasis, blood mained constant on this side but decreased about 40 per cent on the other side. lidocaine was applied to the side of stasis, blood flow remained constant on both When lidocaine was applied to the opposite side, blood flow decreased about 40 per the side of stasis but remained constant on the other side.

eraemia due to injection and due to the induced sympathetic blockade was delimited side of application. This indicates that the two areas investigated are separated by eld at the microcirculatory level. This indicates that the increase in transmural re induced on the side of stasis is not transmitted to the other side.

decrease in blood flow during venous stasis most likely is due to an increase in tension of vascular smooth muscles, as the response is blocked by lidocaine and tamine. Phentolamine applied to the side of stasis does not block the response on the side, which indicates that diffusion of catecholamines from the side of stasis to the side is not responsible. Lidocaine applicated on the side of stasis block the response th sides. This indicates that a vasoconstrictor stimulus elicited by venous stasis is itted to the other side by means of local nerves distributed in an area on both sides of hield. That application of lidocaine on the opposite side blocks the response here s ith this conclusion.

nce the vasoconstrictor response to increase in venous pressure most likely is due to a nervous mechanism involving adrenergic nerves.

D 6

ating Adaptation of Excitable Cells on Analog Computer

By M. COLDING-JØRGENSEN *Institute f Medical Physiology Dept. A, University of Copenhage Denmark*

activity of excitable cells can be considered to be governed by two mechanisms, one onsible for the generation of the spike, and another responsible for the adaptation of cell by ontrolling the firing period. This activity is simulated on an analog computer thising the two mechanisms as follows.

he spike generating mechanism is a simplification of the model presented by Hodgkin Huxley (1952). The sodium and potassium conductances depend on the parameters m nd n in the first pow r with a fixed time constant, and their steady state values are ramp ctions of the membrane potential. The model is able to display excitability and action entials resembling those of the original model

The firing interval is controlled by an extra potassium conductance (adaptation conductance) g_A with the time dependency

$$dg_A/dt=(G_A-g_A)/\tau$$

where the constant τ is much greater than the other time constants of the membra[ne] depends in general on the membrane potential but two extremes can be extracted. [In the] first G_A is zero except when the membrane is depolarised during a spike and g_A therefore only increase when a spike is elicited why the adaptation is called i[mpulse] dependent In the other extreme G_A depends on the potential shift caused by an [...] stimulus as a ramp function of the stimulus. This type of adaptation is called st[imulus] dependent.

The two types are shown to behave quite differently The impulse dependent type [...] to fire continuously at low frequencies and the relation between the magnitud[e of a] constant stimulus and the resulting frequency can be linear over several decades. Thi[s type] behaves like a tonic receptor

The behavior of the stimulus dependent type depends on the function G_A With a [...] slope of the ramp continuous firing is possible for large constant stimuli but not for [...] The relation between stimulus strength and firing frequency is nonlinear and the a[...] resembles the activity of motorneurones as presented by Granit (1972) With a large [...] sustained firing is impossible and the membrane will stop firing after a while even fo[r ...] stimuli. This type behaves as a phasic receptor

It can be concluded that the presented model with one or both of the adaptation [...] can stimulate essential properties of excitable cells

References

Granit R. *The Sherrington Lectures XI* Liverpool 1972.
Hodgkin A L. and A. F Huxley *J Physiol* 1952 *117* 500–544

D 7

Membrane Noise in Receptor Neurons

By L Sjölin and W Grampp *Department of Physiology and Biophysics Univ[ersity] of Lund Sweden*

The slowly adapting stretch receptor neurone of lobster exhibits under subthre[shold] conditions a membrane noise which grows with increasing depolarization and disappe[ars in] response to hyperpolarization beyond resting potential This noise is resistant to 2[...] TEA but can be abolished by 120 nM TTX or by complete substitution of choline or t[...] extracellular Na These circumstances point to the Na-system as the principal [...] source.

For a further study of the noise generating mechanism the noise was recorded as c[urrent] noise under voltage clamp at various potential levels and analysed with respe[ct to] covariance function and spectral density From this analysis it became apparent 1) th[at the] process underlying noise generation obeys first-order kinetics and 2) that the time

the process increases from about 1 ms to about 8 ms, as the membrane potential is from its resting level to firing threshold. This identifies the noise generating as the Na-inactivating process (Hodgkin and Huxley 1952). On the other hand the in noise ariance with depolarization is largely due to an increasing Na-activation, ects of hich remain unrevealed by the present measurements because of the bandwidth (0–250 Hz) of the recording equipment. Thus the recorded current noise from random state transitions of the Na-inactivating system operating on channels pen to be opened by the activating system. In this connexion the depolarization nt increase in time constant of the inactivating process indicates that the inactivat em is not turned on much under subthreshold conditions. This provides for a small y for accomodation which may be one reason for the cells non-adapting mode of n support of this assumption the fact may be quoted that in the fast adapting stretch r neurone it has been imposuble so far to establish the existence of a specific ate noise.

der to see whether noise generation was localized to any particular cell region TTX plied locally to the initial segment of the axon. This resulted in a decrease of the riance as well as in a reduction of the time constant of the noise generating process ggests the possibility of a high density of non-accommodating Na-channels in the of the spike trigger region of the cell.

Reference

N A. L. and A. F. HUXLEY J. Physiol. (Lond.) 1952 117 500–544.

D 8

tion of Pulmonary Arterial Compliance from Registrations of Oscillating Blood Flow essure

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ration of cyclic variations of blood flow and pressure in the pulmonary artery (PA) les the basis for calculation of input impedance of the pulmonary vascular bed. lance is dependent on properties of blood on ascular dimensions and on essel ance. Measurement of impedance may therefore give more information of the lar system than conventional resistance measurement. Since vascular parameters compliance) influence vascular impedance in a rather complex way changes in lance give no obvious information of changes in the vascular system. It may be ble howe er to estimate vessel compliance by the use of mathematical analogs of the lar system requiring identical impedance alues of the analog and the vascular m. The simplest possible analog of the pulmonary arterial system, which takes vessel icity into account, s a lumped compliance, here denoted "effective arterial comce paralleled by arterial resistance. Based on *determination of PA input impedance* and phase difference between pressure and flow oscillations (Ø) at heart frequency (f), ectiv arterial compliance (C_{ea}) is calculated from:

$$C_{ea} = -\sin Ø / 2\pi\ f\ Z$$

This method was preliminarily validated in a computer model of the pulmonary arterial system of rabbits comprising vessels down to 50 μm diameter Arterial compliance in the computer model could be changed by simulating altered myogenic tone of the vessel walls. Input impedance of the computer model was used to calculate C_{eq} and a highly positive correlation (r=0 99) was found between C_{eq} and total arterial compliance in the computer model

The method was used to calculate changes in pulmonary arterial compliance during stimulation of sympathetic nerves to cat lungs During such stimulation C_{eq} was reduced by 60% from the control value (from 2 4 to 0 9×10 ⁸ cm⁵/dyne p<0 01) which is in accordance with reported changes in pulmonary arterial compliance of dog lungs (Ingram et al 1968)

At the present time the method seems to be useful for detecting and quantifying changes in compliance of the pulmonary arterial bed

Reference

INGRAM, R. H J P SZIDON R. SKALAK and A P FISHMAN, Circ Res 1968 22 801–815.

D 9

Phenylethanolamine-N-methyl Transferase (PNMT) Activity in Sympathetic Ganglia of the Cod

By T ABRAHAMSSON and S NILSSON *Department of Zoophysiology University of Goteborg Sweden*

The content of both adrenaline and noradrenaline in the cod spleen decreases significantly after sympathetic nerve sectioning or injection of reserpine or 6-hydroxydopamine (Abrahamsson and Nilsson 1975) which suggests that both amines are stored in the nerve fibres and thus may act as transmitter substances Adrenaline is the dominating catecholamine in the cod spleen while in an other adrenergically innervated organ, the swimbladder muscularis mucosae noradrenaline dominates (Euler and Fänge 1961). This indicates that adrenergic fibres of the same animal have a different catecholamine dominating in the nerve fibres of different organs There is a possibility that in the cod adrenaline synthesized in chromaffin tissue of the head kidney and released into the blood, is taken up and stored in adrenergic fibres A difference in blood supply to the organs could explain the differences in adrenaline/noradrenaline ratio Among other vertebrates, adrenaline has been found to be the dominating autonomic adrenergic transmitter in amphibians (Burnstock 1969)

In the present study the activity of PNMT in the head kidney and the sympathetic ganglia/splanchnic nerve was analyzed to elucidate whether a local synthesis of adrenaline from noradrenaline can take place in the sympathetic neurons of the cod *Gadus morhua*.

To analyze the enzymatic activity of PNMT the method of Axelrod (1962) partly as modified by Mazeaud (1971) was used The synthesis of metanephrine from normetanephrine was identified by paper chromatography The results show a PNMT activity in both the head kidney 1 19±0 16 U/g (n=3) and in the sympathetic neurons

h (n=9) (means ±S.E.). One unit (U) equals 1 nmol of metanephrine formed
anephrine per g tissue per hr. In the sympathetic neurons the differences in
tivity in the sample and blank were tested statistically (t-test for paired values)
ficant differences (p<0.001).

nce of PNMT in the sympathetic neurons indicate a local synthesis of
from noradrenaline in these. This can be taken as further evidence that
together with noradrenaline, is acting as transmitter substance in adrenergic
ne organs of the cod.

References

n, T. and S. Nilsson, Comp. Biochem. Physiol. 1975. In press.
J. Biol. Chem. 1962. 237. 1657–1660.
O., Pharm. Rev. 1969. 21. 247–324.
son and R. Fänge, Gen. Comp. Endocrinol. 1961. 1. 191–194.
L., Thesis, Faculty of Science, Paris 1971

D 10

Nerv. Control of the Dogfish Spleen

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malian spleen is supplied by postganglionic sympathetic fibres which are mainly
, although sympathetic cholinergic fibres have been demonstrated (see review
and Withrington 1973).

re few reports on the effects of drugs and nerve control of the fish spleen. The
a teleost, *Gadus morhua*, releases erythrocytes when stimulated (Nilsson and
74), while Opdyke and Opdyke (1971) concluded that the elasmobranch (*Squalus*
) splee has not developed this capacity.

ork was made to elucidate the effects of some adrenergic and cholinergic drugs
e stimulation on the perfused dogfish (*Scyliorhinus canicula* and *Squalus*
) spleen, and on isolated spleen strips and artery strips from these species.

erfused spleen from either species constricted in response to adrenaline
aline and phenylephrine thereby releasing red blood cells at the beginning of each
ent. Isoprenaline in low doses caused small splenodilatation, which was reversed
triction when higher doses were applied. Isolated spleen strips contracted in
e to the alpha adrenoceptor agonists and so did strips from the coeliac or lieno-
artery. Isoprenaline had no effect on the spleen strips from either species but
the artery strips in the concentration range 10^{-}–10^{-6} M, while higher concentra-
roduced contraction.

ylcholine had very irregular effects on the perfused preparations, and the responses
decreased rapidly due to desensitization of the preparation. The spleen strips from
hinus did not respond at all to acetylcholine, while those from *Squalus* usually

contracted weakly The artery strips from both species however responded well to acetylcholine giving proper dose-response curves

Reproducible effects of electrical stimulation of the splenic nerves in the perfused spleen preparation were seen in *Squalus* only The constriction obtained during stimulation could be abolished by relatively low concentrations of phentolamine (10^{-7} M) present in the perfusion fluid while atropine (up to 10^{-5} M) caused an enhancement of the nerve response Mecamylamine (10^{-4} M) had no detectable effect on the nerve response.

It is concluded that the *Squalus* spleen is regulated primarily by adrenergic fibres. Presence of a cholinergic link in the nervous pathways to the different effectors in the spleen (vessels trabeculae capsule) is possible but could not be detected with certainty in the present work. The *Scyliorhinus* spleen may be regulated to a large extent by circulating catecholamines since the nerve supply appears to be poor Changes in plasma concentrations of adrenaline and noradrenaline during stress is within the range where we find contraction of isolated spleen strips or artery strips occur (Mazeaud 1969)

References

DAVIES, B N and P G WITHRINGTON *Pharm Rev* 1973 *25* 373–413.
MAZEAUD M *C R Séanc Soc Biol* 1969 *163* 2262–2266.
NILSSON, S and D J GROVE, *Eur J Pharmacol* 1974 *28* 135–143
OPDYKE, D F and N E. OPDYKE, *Amer J Physiol* 1971 *221* 623–625

D 11

Drug Effects on Gas Production and Smooth Muscles in a Fish Swimbladder

By R FÄNGE, S HOLMGREN and S NILSSON *Department of Zoophysiology University of Göteborg Sweden*

The swimbladder of the wrasse (*Ctenolabrus rupestris*) is a closed cavity which is divided into an anterior and a posterior part by a transverse diaphragm with a central hole. The swimbladder is filled by secretion of mainly oxygen from the flattened reddish gas gland in the ventral part of the anterior portion. During gas secretion the diaphragm moves backwards by movements in the smooth muscle of the mucosa, thereby enlarging the anterior or secretory part considerably The gas is resorbed when the secretory part contracts and thereby moves the diaphragm forward and enlarges the thin, gaspermeable posterior or resorbent part (Fänge 1953 1973)

Responses of strips from the anterior or posterior parts of the mucosa to different drugs were recorded as cumulative dose-response curves. The anterior part contracts in response to the adrenergic agonists adrenaline noradrenaline and phenylephrine and to acetylcholine and 5-hydroxytryptamine while isoprenaline has no effects The order of potency of the adrenergic agonists and the fact that phentolamine produces a parallel shift of the noradrenaline dose-response curve indicates the presence of alpha adrenoceptors. The posterior part contracts in response to acetylcholine. Strips precontracted with acetylcholine relax by addition of isoprenaline adrenaline and noradrenaline and the relaxation is competitively antagonized by propranolol indicating that the effects are due to

of beta adrenoceptors. Precontracted strips are also relaxed by ytryptamine.

al of 0.5 ml of the swimbladder gas stimulates gas secretion from the gas gland. In jected by atropine or mecamylamine immediately after removal of the gas, the production is significantly inhibited. No significant alterations in gas secretion after of gas is produced by reserpine, yohimbine or 6-hydroxydopamine. However in which have not been subject to gas removal reserpine yohimbine or ydopamine cause a backward movement of the diaphragm increasing the area of tory part.

escent histochemistry reveals smooth and varicose adrenergic fibres in both parts uscularis mucosae. Yellow fluorescent cells, similar to those described in *Salmo* et al. 1965), which may contain 5-hydroxytryptamine, are also seen

concluded that a cholinergic pathway may be required for gas secretion in ions. A considerable part of the nervous control of the muscularis mucosae may be adrenergic fibres, which act *via* alpha adrenoceptors in the anterior part and beta ceptors in the posterior part.

References

O., B. FALCK and E. ROSENGREN. *Acta physiol. scand.* 1965. 64. 119–126.

R. *Acta physiol. scand.* 1953. 30. Suppl. 110. 1–133.

R. In *Comparative Physiology*. Ed. L. Bolis, K. Schmidt-Nielsen and S. H. P. Maddrell. 1973 159.

D 12

hine for On Line Calculation of Time Interval and Peak Amplitude Histograms

y L. SJÖLIN *Department of Physiology and Biophysics. University of Lund. Sweden*

logical experiments are normally characterized by the accumulation of large ties of raw data. Manual processing of these data is often tedious and time consum- d can only be performed off line. Often this may result in unnecessary repetition of periment.

connexion with studies of Schwann cell miniature end plate potentials (min. e p p.s.) ing denervation of frog skeletal muscle (Birks, Katz and Miledi 1960), it was felt that effort in data processing would be saved if a digital handling system that could ate peak amplitude or time interval histograms was constructed. The time interval should include the determination of latency time, time between successive events, se time

e signal to be processed in the peak amplitude mode is passed on to an analog to al converter so designed that it determines the peak value of the min. e.p.p. and erts it into an eight bit binary word. In time interval measurements the elapsed time is erted into an eight bit binary word. This word represents a certain address within a nory array (256 memory cells, each containing an eight bit word). Whenever a certain nory cell is addressed by the peak detector its content is increased by one. At the time

when the content of any cell reaches 240 the memory is disabled Thus considering t the measured peak voltage or time interval corresponds to an eight bit binary wo voltage levels or time intervals containing a maximum of 240 counts can be presente histogram is continuously displayed by means of a step generator that sequentially a ses all the memory cells The address signal is digital to analog converted and fed x-amplifier of a cathode ray oscilloscope The eight bit binary word that represe content of a cell is likewise converted to an analog signal and displayed vertically oscilloscope

The machine utilises mainly TTL logic circuitry and the total costs amount to 150£.

Reference

BIRKS, R., B KATZ and R MILEDI, *J Physiol* (Lond.) 1960 *150*, 145–168.

D 13

Actions of Local Anæsthetics Applied by Microiontophoresis to Motoneurones

By I ENGBERG, J A FLATMAN K KADZIELAWA and J D C LAMBERT *Depart of Physiology Aarhus University Denmark*

Several amines and adrenoceptor blockers cause hyperpolarization and increase the a potentials when applied microelectrophoretically to motoneurones (Engberg Flatmar Kadzielawa 1974) Some adrenoceptor blockers possess a local anæsthetic actio excitable tissues causing a block of the spike generation mechanism.

The classical local anæsthetic agents share a similar structure to the monoamine agonists and antagonists, a terminal ring a short intermediary chain and a terminal a group An investigation of the action of amethocaine procaine and lignocaine was un taken to characterize local anæsthetic action on cat spinal motoneurones and to extend earlier investigations of Curtis and Phillis (1960)

The local anæsthetics were found to produce a rapid hyperpolarization with membr resistance increase and increase of the spike overshoot all of which corresponded to typical amine responses However towards the end of longlasting drug applicatio spikes evoked by intracellular current injection were reduced in amplitude, and antidro spikes were blocked On cessation of amethocaine applications a further marked reducti and prolongation of the spike was seen together with a small membrane depolarizati The maximal action occurred up to 3 min after drug application, and a further five minu lapsed before the spike and the membrane potential returned to normal A similar b smaller and more rapidly reversible action was observed with procaine and lignocaine a with the adrenoceptor blockers pindolol and thymoxamine Interestingly noradrenali partially restored spikes reduced by amethocaine

Amethocaine is more lipid soluble and more potent a local anæsthetic than the oth agents. This was probably reflected by its comparatively strong and longlasting action c the spike Similarly amethocaine has a slower and more prolonged action than the oth local anæsthetics at the frog node of Ranvier (Flatman 1968)

In conclusion the local anæsthetics showed two separate actions Firstly a membran

ization with increase of spike overshoot and membrane resistance. Secondly a reasing block of the spike after the cessation of the iontophoretic current and the the membrane potential to its resting level

References

R, and J W Phillis. *J Physiol.* 1960 *153* 17–34.

L, J A. Platman, and K. Kadzielawa. *Acta physiol. scand.* 1974, *91* 3 A–4 A.

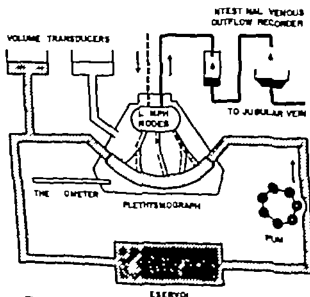
J A Ph D Thesis, University of London.

D 14

mographic Method for Continuous Determination of Net Water Transport across inal Epithelium in vivo

Mats Jodal, Dan-Axel Hallbäck, Joar Svanvik and Ove Lundgren. *Department of Physiology University of Göteborg Sweden*

net water transport is usually measured *in vivo* by means of an unabsorbable g polyethylene glycol (PEG). Since transient changes occurring within minutes not detectable we have developed a method enabling such studies in tized cats (see Fig). An intestinal segment is enclosed *in situ* in a perspex ograph, the intestinal lumen being steadily perfused with a solution of known ion from a closed perfusion system with a reservoir large enough to prevent tion. Motility can be detected by direct inspection and by recording simultaneously large but opposite volume changes in the two volume transducers. If no



motility occurs the change of the perfusion volume reflects net water transport across the intestinal epithelium A quantitative comparison of this technique with the conventional PEG-method revealed no significant difference

The present technique does not only allow a quantitative determination of total water absorption but also an estimation of the fluid amount absorbed *via* the lymph. Thus control experiments show that this latter volume is recorded as a continuous increase of intestinal volume if it is assumed that net fluid exchange occurs only across the absorbing intestinal capillaries The fluid absorbed into the lymph is "trapped" in the plethysmograph since it cannot overcome the high sealing pressure at the exit. When excluding the left volume transducer from the closed luminal perfusion system a decrease of plethysmographic volume is recorded which reflects net water absorption of fluid *via* the blood The amount of fluid absorbed from the perfusion system can now be derived from its sole compliant part *i e* the intestine and fluid absorbed *via* the lymph remains in the plethysmograph

D 15

Anaerobic Glycogen Metabolism in the Ampullariid Snail *Marisa cornuarietis*

By G ÅKERLUND. *Department of Zoology Univ of Gothenburg Sweden*

The ability of molluscs to survive anaerobic conditions has been examined by several authors However this property varies between different groups of molluscs Thus bivalvia have been shown to withstand stressful oxygen free conditions for several days while many freshwater snails are incapable of surviving more than a few hours of anaerobiosis. Bivalvia do not accumulate lactic acid during anoxic conditions but alanine and succinic acid The main energy pool during this period is glycogen.

However the ampullariid freshwater snail *Pila virens* which resists drought periods by aestivation has been shown to survive under laboratory conditions for 6 months in dry oxygen free environments This snail metabolizes glycogen during aestivation at a slower rate than during anaerobiosis and accumulates lactic acid

In my investigations I have been studying the anaerobic glycogen breakdown and lactic acid accumulation in the ampullariid *Marisa cornuarietis* This snail is of interest because it might be used as an agent to biological control snails bearing tropical disease bilharzia. The results indicate a rapid decrease of glycogen during anaerobiosis no accumulation of lactic acid and a tolerance of oxygen free conditions for about 16 h

D 16

Correction for Changes in Counting Efficiency during Elimination of ^{133}Xe from Subcutaneous Fat

By JENS BÜLOW and JOOP MADSEN *Medicinsk-fysiologisk institut C Copenhagen*

When adipose tissue blood flow (ATBF) is determined from the rate constant for elimination of an injected ^{133}Xe depot (Larsen *et al* 1966), it is important that the subject lies immobile Otherwise the elimination curve become irregular because of changed counting

metry. Consequently it has not been possible so far to determine ATBF during heavy ...tion. To evade this problem the following procedure was developed.

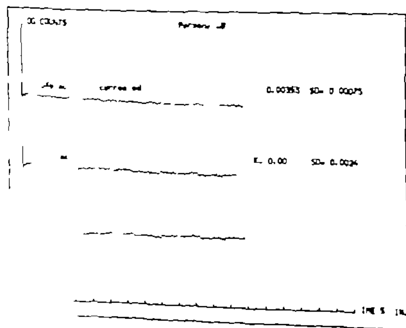
The position of the subcutaneous ^{133}*Xe depot is carefully* marked on the skin. At this site ...dioactive γ-source (^{99m}Tc or ^{57}Co) is placed by mean of adhesive tape. The activity is ...sured by a scintillation detector placed at some distance from the activity. The ...tector is connected to a 2 channel discriminator one channel set for the 81 KeV ^{133}Xe-peak, the other for ^{99m}Tc or ^{57}Co. After correction for background, resolving time, ...sical decay and the contribution of ^{133}Xe in the ^{99m}Tc (or ^{57}Co) channel variations in the ...Tc figures must be caused by changes in counting efficiency (geometry) affecting the ^{133}Xe counts in a similar way. The ^{133}Xe figures were corrected for the same determinate ...ors as ^{99m}Rc and were finally corrected for changes in counting efficiency as follows

$$^{133}Xe_{corrected} = {}^{133}Xe_n \cdot \frac{^{99m}Tc_{initial}}{^{99m}Tc_n}$$

Fig. 1 demonstrates the effect of the correction procedure in a bicycle ergometer ...periment. In 7 expts on supine restless subjects, the standard deviation of the rate ...stant was reduced by 40–50 per cent at an average. *Thus the procedure increase the* ...sibility of proving small changes in ATBF

References

...sen, O. A., N. A. Lassen and F. Quaade, *Acta physiol. scand.* 1966, 66, 337–345



...g. 1. Wash-out of ^{133}Xe from depot in lumbar subcutaneous fat during work on bicycle ergometer. A ...Tc source was placed on the skin over the ^{133}Xe depot. Distance to detector: 70 cm. Registration ...very 20 s. Ordinate. The distance between the intercepts corresponds to one decade.

D 17

The Effect of Alanine on Cell Membrane Potentials in Rat Liver

By M FOLKE and M PALOHEIMO. *Institute of Medical Physiology Dept A, versity of Copenhagen Juliane Mariesvej 28 DK 2100 Copenhagen Denmark*

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The initial depolarization is compatible with the hypothesis that alanine may be take into the liver cells by a Na -coupled transport mechanism. If this hypothesis is tr likely explanation for the subsequent hyperpolarization would be electrogenic extrusi Na from the cells by Na –K activated ATPase. In our current experiments t possibilities are investigated

References

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D 18

The Disappearance of Hydrogen Ions from the Normal Unstimulated Stomach

By BERTIL FRENNING *Departments of Internal Medicine and Physiology and Med Biophysics, University of Uppsala, Sweden*

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Reference

Fr..., B., *Upsala J. Med. Sci.* 1972, Suppl 11

D 19

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Ex...

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In a control period 6 ml of 170 mM hydrochloric acid was instilled into the chamber The instillate was stirred continuously After a 30 min period the instillate was removed. Then 6 ml of 170 mM hydrochloric acid was repeatedly instilled during 30 min periods but during these periods physiological saline was infused into the instillate by an accurate infusion pump at a rate of 1 5 3 or 4.5 ml/30 min, respectively The net flux of hydrogen ions during the different periods was determined. During the control period there was a mean loss of 208 ± 17 μEq/30 min (±S.E., n = 5). When saline was infused at a rate of 1.5 ml/30 min the loss of hydrogen ions remained at the control level (206 ± 19 μEq/30 min) but

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...no, B., *Upsala J. Med. Sci.* 1972. Suppl 13.

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...y B. Frenning and P. G. Öhrn. *Departments of Internal Medicine and Clinical Re...earch II University Hospital, Uppsala, Sweden*

...ure of the gastric mucosa to acetylsalicylic acid (ASA) increases its permeability to ...and to macromolecules. Subsequent exposure to hydrochloric acid or cholinergic ...lation causes hemorrhage and mucosal erosions. In man, an association between ...late intake and gastric ulcers has been suggested. The mechanism behind the effects ...SA is suggested to be an intracellular accumulation of the drug causing increased ...cellular pathways in the mucosa. (For references see Frenning 1972.) It has also been ...sted that reflex of bile containing duodenal contents into the stomach may contribute ...t salicylate induced mucosal damage (Djahanguiri *et al.* 1973). The possible effect of ...on gastric emptying and small intestinal propulsion was studied in rats previously ...ided with permanent gastric and duodenal tubes permitting studies on unanaesthetized ...als. The stomach was exposed to 1.5 ml of 10 mM ASA dissolved in 100 mM HCl ...aCl or to 1.5 ml of 100 mM HCl. After 30 min radioactive test meals were given. Intra...ically 1 ml of ^{131}I labelled polyvinylpyrrolidone solution (PVP) was given and intra...denally 0.2 ml ^{125}I labelled PVP solution. After 15 min the animals were killed and ...ures placed at the cardia, the pylorus and the caecum. The specimen was removed in ...inuity and stretched out on a perspex plate. The radioactivity was then detected with ...amma detector according to Nilsson *et al.* 1973. After 15 min the stomachs exposed to ...dissolved in 100 mM HCl had evacuated 79.6 ± 5.8 (± S.E., n = 5) per cent of the radio...ve test meal. The corresponding figures for the stomachs exposed to ASA dissolved in ...mM NaCl was 80.1 5.3 (6) and for the control group 87.6 ± 1.6 (n 6). No ^{125}I ...ld be detected in the stomach in any of the animals (i.e. no duodeno–gastric reflux oc...ed). The per cent of small bowel length passed by 50 per cent of the activity deposited ...he duodenum was 63.8 2.5 in the animals exposed to ASA dissolved in 100 mM HCl, ...3.4 in the animals exposed to ASA dissolved in 100 mM NaCl and 67.2 ± 1.5 in ...control animals. There was no statistically significant difference between any of the

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References

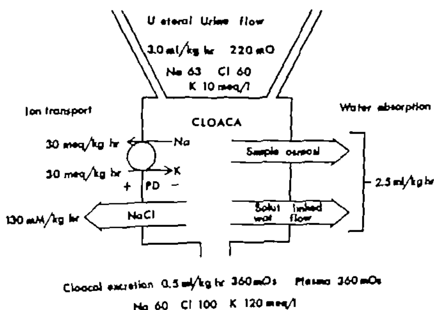
DJAHANGUIRI B F S. ARTAIG and M HEMMATI, *Gastroenterology* 1973 *65* 630–633
FRENNING, B., *Upsala J med Sci.* 1972. Suppl. 13
NILSSON, P B. LJUNG and H LUNDQVIST *Act chir scand.* 1973 *139* 716–723

D 20

Cloacal Absorption in the Lizard *Agama stellio*

By I DUVDEVANI † and E. SKADHAUGE. *Department of Zoology University of Tel Aviv and Institute of Medical Physiology A University of Copenhagen*

The absorption of NaCl and water in the coprodeum of the xerophilic lizard *Agama stellio*, and the secretion of K was evaluated by *in vivo* perfusion of the coprodeum in normally hydrated, in dehydrated and in water and salt loaded animals. The ionic and osmotic concentration of ureteral urine, of plasma, and of the cloacal contents was measured. In the normally hydrated state the ureteral urine had an osmotic urine to plasma ratio of 0.7 and it came into osmotic equilibrium in the coprodeum. Perfusion fluids of the osmolality of ureteral urine was used In normally hydrated animals the Na and Cl absorption rates were 203 and 131 μeq/kg.h respectively the secretion rate of K was 45 μeq/kg.h, and the water absorption 2.4 ml/kg.h This represents a resorption at approximately 80% of the ureterally excreted NaCl and water In *Fig 1* the cloacal treatment of salt and water is



Cloacal excretion 0.5 ml/kg hr 360 mOs Plasma 360 mOs
Na 60 Cl 100 K 120 meq/l

...ated using rounded values for ureteral and cloacal excretion rates. Since the apparent ...olality of the absorbate is only 109 mOs, the water absorption is partly due to simple ...osis, partly to a solute-linked water flow. The types of osmotic stress used all resulted ... changes of transport rates in directions which would serve the osmoregulatory needs of ... animal.

D 21

...pe II Fibres in Human Skeletal Muscle: Biochemical Characteristics and Distribution

By E. Jansson, *Department of Clinical Physiology, Karolinska Hospital, Stockholm, Sweden*

...man skeletal muscle contains two major fibre types, type I and type II, based on myo...rillar ATPase stain (Padykula and Herman, 1955). Brooke and Kaiser (1970) have with ...odifications of this stain, preincubations at different acid and alkaline pHs found 3 sub...oups of the type II fibre (IIA, IIB and IIC), which might indicate the existence of ...fferent kinds of myosin. The fibres also differ in oxidative and glycolytic capacities as ...dged from histochemical staining procedures. In order to evaluate and quantify this ...pposed metabolic difference single muscle fibres were dissected out, classified according ... Essén et al. and analysed with regard to succinatedehydrogenase (SDH), an oxidative ...zyme in Krebs' cycle and phosphofructokinase (PFK) a regulatory enzyme in the glyco...sis. The results (Fig. 1) reveal that the type IIB fibre has the lowest SDH activity and ...C the highest, almost as high as the activity in type I. PFK shows the opposite pattern ...th the highest activity in the type IIB fibre. Consequently these findings are in agree...ent ...ith the previous histochemical estimate.

...We have further investigated the percentage distribution of the different subgroups of ...e type II fibres in untrained and extremely welltrained (endurance) individuals. The most ...teresting finding was that in the majority of the welltrained subjects no IIB fibres were ...und in vastus lateralis or gastrocnemius whereas the untrained had between 8 and 25%, ...ean 14 .... It is at present not possible to tell if the extreme training has caused a change

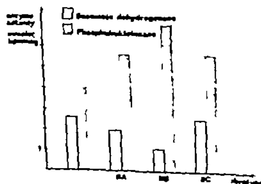


Fig. 1 Enzyme activities in individual muscle fibres. The bars represent the ... mean of determination of 3 fibres.

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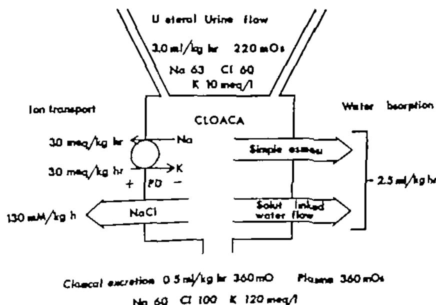
DJAHANGUIRI, B., F. S. ABTAHI and M. HEMMATI, *Gastroenterology* 1973. 65 630–633.
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presented using rounded values for ureteral and cloacal excretion rates. Since the apparent osmolality of the absorbate is only 109 mOs, the water absorption is partly due to simple osmosis, partly to a solute-linked water flow. The types of osmotic stress used all resulted in changes of transport rates in directions which would serve the osmoregulatory needs of the animal.

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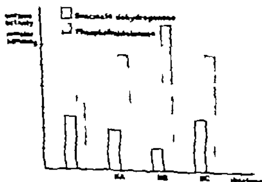


Fig. 1. Enzyme activities in individual muscle fibres. The bars represent the mean of determination of 5 fibres.

in fibre composition with the less oxidative IIB fibre converted to more oxidative IIA and a change in myosin molecule configuration, which in turn might indicate altered contraction characteristics.

References

BROOKE, M H. and K. K KAISER, *Arch. Neurol. (Chicago)* 1970. *23* 369–379
ESSÉN, B., E. JANSSON J HENRIKSSON, A. W TAYLOR and B. SALTIN, Metabolic characteristics of fibre types in human skeletal muscle. Under publication.
PADYKULA H A. and E. HERMAN, *J Histochem Cytochem.* 1955 *3* 170–195.

D 22

Reactant Introduction in the Course of Assay of Oxygen Consumption Performed by Microspectrophotorespirometry

By R HULTBORN and T LICHTNECKERT *Institute of Neurobiology University of Göteborg Sweden*

A microrespirometric system for analysis of O_2-uptake of small tissue samples or single cells *in vitro* was developed by Hultborn (1974). The material to be analysed is incubated, with a medium including oxyhemoglobin, within minute (1 µl to 10^{-4} µl) chambers (cuvets) (*Fig.* 1) and respiration is assayed by recording the dissociation of the oxyhemoglobin due to the decreased O_2-tension caused by cellular oxygen extraction. The absorbance change of the incubate at 435 nm is recorded using a microscopespectrophotometer The procedure has proved to be sensitive (down to 10^{-6} µl O_2/h), accurate and convenient (Hultborn 1974).

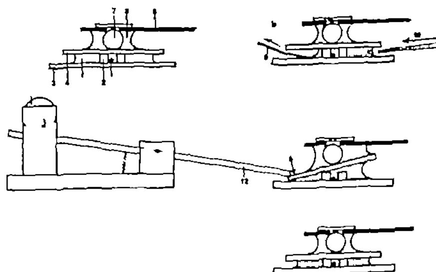


Fig. 1 ( ) 1 tissue within cuvet, 2 cuvet wall, 3. plane glass slide, 4 cover glass, 5 medium surrounding cuvet, 6. steel clamp, 7 glass sphere fitted in the clamp t ascertain centric pressure, 8. immersion oil to avoid light refraction. (b) Replacement of medium outside the cuvet 9 filter paper 10. pipette. ( ) Tilting of cover glass by a lever 12, operated by an eccentric wheel 11 (*d*) Assay starting from the beginning with the reactant introduced.

In many metabolic studies the absolute respiratory rate is not crucial, but rather the ...ge of the tissue on addition of a reactant, e.g. a hormone. Further it is in many ... especially when the sample is very small, difficult to relate the O_2-uptake to some ...onal parameter of the object (e.g. weight). It is in this case necessary to be able to ... the metabolic activity of the same sample both before and after addition of the re...t, the effect of which is to be studied.

...imple procedure fulfilling this demand has been developed for the spectrophoto...metric procedure. The glass covering the cuvet (see Fig.) has been extended so that ... be tilted by a lever connected to an excentric wheel, which is moved either by hand ... a motor. Respiration under basal conditions is allowed to proceed to 50% O_2-satura... The medium outside the cuvet is then replaced by the new one, repetetive tilting ...sec/20 times) is commenced. Almost complete (95%) exchange of medium in the cuvet ... place and assay of respiratory rate starts from the beginning. The slopes of the ...rves before and after reactant introduction are compared.

...o the problem of biological variation will be greatly diminished by comparing the ...ns of the same sample to different reactants.

Reference

...ers, R., Acta physiol. scand. 1974, Suppl. 404, 1–47

D 23

Exchange of 3-O-Methylglucose in Isolated Fat Cells. Concentration dependence ... effect of insulin

By J. Vinten and J. Gliemann. *The Institute of Medical Physiology C. University of Copenhagen, Denmark*

...self exchange of D-3-O-methylglucose was studied using a modification of the oil ...tion technique described by Gliemann *et al.* (197?). It was found that the sugar equili...tes over the membrane, and that insulin is without effect on the magnitude of the distri...ion space at equilibrium. The disappearance of intracellular ^{14}C-labelled D-3-O-methyl...cose under conditions of self exchange could be described as an exponential ... (Fig. 1 left). In the presence of insulin (7 · $10^{-?}$ M), the exchange rate was increased ...5 times, whereas the presence of phloridzin (5 mM) reduced the exchange rate at a ...-M-glucose conc. of 50 mM about 20 times. Fig. 1 (right) shows the half time for the ...hange process as a function of the sugar concentration, both in the absence and in ... presence of insulin. The apparent half saturation constant for the exchange process is ...out 5–10 mM for both curves, whereas the transport capacity is increased about 25 times ... insulin. Experiments on the inhibition by 3-O-methylglucose of the incorporation of ...ce amounts of U-^{14}C-glucose into intracellular lipids confirmed this finding. It is thus ...ncluded, that the insulin action on the sugar transfer mechanism is to increase its ...nsport capacity without changing its affinity to the sugar.

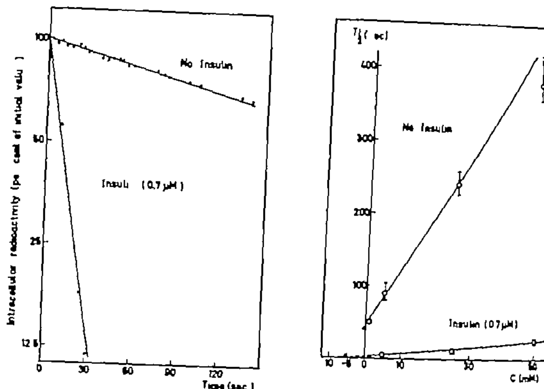


Fig. 1 The effect of insulin on exchange for 3-O-methylglucose. Left The cells were preincubated for 30 min with 25 mM of 3-O-methylglucose and 5 μCi/ml of 3-O-[14C]methylglucose in the absence and in the presence of insulin. The cells were centrifuged through silicone oil (specific gravity 0.97) and at zero time dispersed into a medium containing 25 mM of unlabelled 3-O-methylglucose. Right. The half-times of 3-O-methylglucose exchange in the absence and in the presence of insulin are plotted as a function of the 3-O-methylglucose concentration. Each point represents one efflux curve ± SE.

Reference

Gliemann, J K Österlind J Vinten and S Gammeltoft *Biochim. Biophys. Acta* 1972, 286, 1 9

D 24

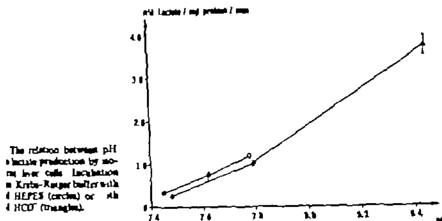
The Influence of pH on the Lactate Production by Isolated Parenchymal Rat Liver Cells

By O Jalling and C Olsen *Institute of Physiology University of Aarhus, Denmark*

Elevated concentrations of lactate in the blood are consistently seen in connection with a rise in extracellular pH (Oliva 1970) The production as well as the removal of lactate seems to be involved In respiratory alkalosis the normal hepatic uptake of lactate was reversed to an output, this effect was ascribed to changes in the circulation (Berry and Scheuer 1967)

The aim of the present study was to test the effect of changes in the extracellular pH HCO_3^- and P_{CO_2} on the lactate production of isolated parenchymal liver cells.

The cells were prepared by the method of Quistorff et al. (1973). Starved rats were used. 10 mM glycerol was the substrate. 3 series of experiments were performed. The cells were incubated at 37°C in Krebs-Ringer buffer with A) HEPES 25 mM (n = 6), B) HCO_3 25 mM and P_{CO_2} about 36 16 and 4 mm Hg (n = 6) and C) HCO_3^- 15 25 and 35 mM and P_{CO_2} adjusted to obtain constant pH of about 7 4 (n = 5)



The relation between pH
lactate production by no-
rat liver cells. Incubation
n Krebs-Ringer buffer with
l HEPES (circles) or with
l HCO_3^- (triangles).

e results from series A and B are represented in figure 1 which demonstrates a sig-
ant effect of changes in the extracellular pH on the lactate production by liver cells.
anges in the concentration of HCO_3^- and in P_{CO_2} with constant pH did not significant-
luence the production of lactate which remained about 0.40 nM lactate/mg protein/min.
e results thus demonstrate enhanced output of lactate from the liver cells with rising
acellular pH, whereas changes in the concentration of bicarbonate and in the P_{CO_2}
constant pH had no effect in the range tested.

s the lactate is derived from glycerol, a production of lactate implicates an equivalent
uction of protons. It seems likely therefore that the liver participate in the com-
atory processes both to respiratory and metabolic alkalosis even without changes in
circulation.

References

...r, M. N. and J. Schuler, *Metabolism* 1967 *16*, 537–547
...a, P. B. Amer. J. Med. 1970 *48*, 209–225
...olf, B. S. Bondurn and N. G. ...nney, *Biochim. Biophys. Acta* 1973, *330*, 503–516.

D 25

...lease of a Large Fraction of Oxytocin Stored in Rat Neurohypophyses by Intensive ...mulation *in vitro*

By S. Dalby, E. Engberg, J. T. Russell and N. A. Thorn, *Institute of Medical Physiology C, University of Copenhagen, Denmark*

...vious studies have shown that stimulation *in vitro* of vasopressin release can maximally
...obilize some 10 % of the total hormone content. The existence of an easily releasable
...action of vasopressin has been suggested on the basis of such experiments (Thorn 1966,
...a.b.s *et al.* 1967). To test limitations in the amounts of oxytocin that may be mobilized
in vitro the following experiments were performed

Groups of isolated hemilobes of rat neurohypophyses were stimulated for 90 min with a 56 mM potassium concentration in the surrounding medium, and the release of oxytocin to the medium was measured by an isometric isolated rat mammary gland strip assay

A total of 23% of the oxytocin content was released by this procedure.

When sodium was replaced by sucrose throughout the stimulation period the percentage release increased to 29%. Removal of sodium after 60 min stimulation also caused an increase in the release rate. Removal of calcium from the medium after 10 min of stimulation caused a rapid fall in the release rate. Reintroduction of calcium caused an increase of the release.

Treatment of the isolated neurohypophyses with n-ethyl-maleimide (NEM) (10 mM for 10 min) followed by a 90 min stimulation by 56 mM potassium caused a total release of a 42% of the contents.

When after 40 min stimulation the potassium concentration of the medium was increased to 90 mM an increase in the release took place.

Neurohypophyses which were stimulated repeatedly by 56 mM potassium for 12 min followed by 9 min incubation in control medium responded with at least a fivefold increase in oxytocin release even 210 min after the start of the repetitive stimulation.

Removal of sodium and presence of NEM presumably interfere with the transport processes for Ca   Increasing the concentration of free, intracellular Ca   which is important for release (Russell and Thorn 1975 Thorn 1974).

It is concluded that procedures which potentiate the initial processes in stimulus-secretion coupling may increase the amount of hormone that can be mobilized Limitations in release may be more dependent on limitations in the activation processes than on maturity or localization of the hormone containing granules within the nerve endings.

References

Russell, J T and N A. Thorn Act physiol scand. 1975 (In press).

Sachs, H L. Share, J Osinchak and A. Carpi, *Endocrinology* 1967 *81* 755–770.

Thorn N A *Act endocr* (Kbh) 1966. *53* 644–654

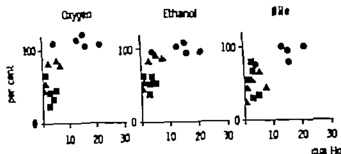
Thorn, N A., Role of calcium in secretory processes. In *Secretory mechanisms of exocrine glands. The Alfred Benzon Symposium VII* (eds. N A Thorn and O H Petersen). Pp. 305–3 6. Munksgaard, Copenhagen, 1974

D 26

Hepatosplanchnic Hemodynamics and Liver Function during Graded Hypoxia

By N Krarup J A Larsen and A Munck *Institute of Physiology University of Aarhus, Denmark*

The hepatosplanchnic vascular response to hypoxia has not yet been definitely established (Greenway and Stark 1971). Further a critical limit of p_{O_2} in hepatic venous blood above which a normal liver function is maintained, is not available from the literature. It was therefore of interest to study the effect of hypoxic hypoxia on the hepatosplanchnic hemodynamics and liver function. 15 chloralose anesthetized, curarized cats were ventilated with atmospheric air to a pa_{CO_2} of 40 mm Hg by means of a Harvard respirator Following



1. The hepatic oxygen consumption rate, splanchnic ethanol elimination rate and the bile flow ...ed as per cent of the control levels, plotted against the hepatic venous p_{O_2} during hypoxemia induced ...athing mixtures of nitrogen and oxygen 10% (■), 12.5% (▲) and 15% (●).

) minutes control period the inspiratory gas was altered to mixtures of nitrogen and 12.5 or 15% oxygen for a further 60 minutes period. 5 experiments were performed ... each mixture of gas. The resulting hypoxemia was followed by immediate increases ...ean arterial blood pressure which, however returned towards control levels during ...ext 10 minutes, when the inspiratory gases contained 15 and 12.5% oxygen. Inspiration ...0% oxygen caused a steady decrease in arterial blood pressure. Except for a 70% in... ...e in gastro-intestinal conductance, when the cats were inspiring 10% oxygen, no changes ...he conductances in the gastrointestinal area, the hepatic artery or in the intrahepatic ... pressure vessels occurred. The splanchnic elimination rate of ethanol, the hepatic ...gen consumption and the bile flow were all affected by the hypoxemia, but, as is appar... from Fig. 1 only when the hepatic venous p_{O_2} became lower than 10 mm Hg. This ...icates that the intrahepatic perfusion is homogeneously distributed, as an uneven distri...tion of blood flow in the exchange vessels in the liver might result in functional disturb...es at higher values of hepatic venous p_{O_2}. The finding of a gradual decrease in liver ...ction in perfused pig livers when hepatic venous p_{O_2} was lowered from 35 mm Hg (Rabol ... al. 1974) may be explained by an uneven perfusion in this preparation.

References

...EDW C. V. and R. STARK, Hepatic vascular bed. *Physiol. Rev.* 1971. *51* 23–65.
...BOL, A. F. V. HANSEN, S. KEIDING, N. TYGSTRUP, K. TONNESEN and K. WINKLER, The effect of hy...oxemia on the function of the isolated perfused pig liver. *Digestion* 1974 *10*. 375–76.

D 27

...yclic AMP Content and Phosphodiesterase Activity in Rat Corpora Lutea of Differ...t Ages

By I. KHAN, S. ROSBERG, H. HERLITZ and K. AHRÉN. *Department of Physiology University of Göteborg, Sweden*

...any experimental findings favour the hypothesis that the stimulatory action of LH on ...rogesterone synthesis by the corpus luteum (CL) is mediated by cyclic AMP (cAMP).

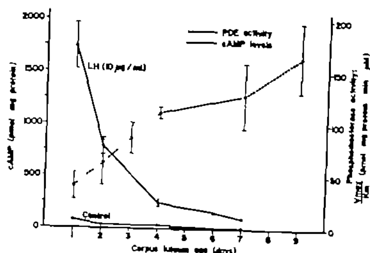


Fig. 1 cAMP and PDE activity in rat corpora lutea of different ages. Corpora lutea (CL) were isolated 1 9 days after ovulation from ovaries of PMSG-injected rats (see text). In one experiment cAMP contents were determined. *CL* were incubated with and without LH (10 µg/ml) for 15 min and analysed for cAMP content as described earlier (Herlitz *et al.* 1974). Mean ± S.E. are shown in the figure (5).

In separate experiments PDE activities were determined. CL were homogenized in Tris buffer (20 mM Tris, 6 mM $Mg(CH_3COO)_2$, pH 7.5) and centrifuged at 2000 *g* for 10 min. Aliquots of the supernatant were mixed with equal volumes of ^{3}H-cAMP (5 different concentrations between 0.1-0.01 µM) and incubated for 15, 30 and 45 *min* at 37°C. *Degradation* rates of ^{3}H-cAMP were estimated according to Pöch (1971). PDE-activity (V_{max}/k_m) was determined in a Lineweaver Burk plot. Mean ± S.E. for n determinations are shown in the figure.

Marsh (1970) studied the effects of LH on the adenylate cyclase (AC) and the phosphodiesterase (PDE) in the bovine CL, and concluded that the effect of LH on progesterone production was due to stimulation of AC and not due to inhibition of PDE. It has, in addition, been shown in some species that stimulation of progesterone synthesis by LH is less effective towards the end of the CL phase. Stansfield *et al* (1971) reported that bovine CL at the stage of regression had an increased PDE activity compared to CL collected earlier during the estrous cycle. These authors investigated, however only the enzyme with the high K_m value.

In our laboratory studies have been undertaken to follow the sensitivity to LH with respect to cAMP formation during the aging of the rat CL (Herlitz *et al* 1974). In these studies, a "PMSG-model" has been used where pregnant mare's serum gonadotrophin (PMSG) has been injected to 30 day old immature rats to cause ovulation and formation of CL early in the morning of day 33. Very young CL (1 day old) show a pronounced sensitivity to LH when cAMP is studied while older CL (7 day old) show a markedly decreased response. Also the control values decreased with increasing CL age.

In the present study the above mentioned observation was correlated to the PDE activity of CL. The PDE activity in homogenates of CL of different ages were determined according to Pöch (1971) using ^{3}H-labelled cAMP. No differences in activities were seen between CL of various ages when the enzyme with the high K_m (100–250 µM) was measured, but the activity of the low K_m enzyme form (0.1–0.2 µM), which seems to be more closely correlated to the physiological intracellular cAMP concentrations in CL, steadily increased with increasing age of CL (Fig. 1). The physiological meaning of this enhanced degradation

of cAMP with increasing age of the CL is not clear but it might be involved in the termination of the luteotrophic effect of LH. Studies are now in progress to elucidate whether the increased PDE activity alone can explain the decreased response to LH which is seen with increasing age of CL when cAMP contents are determined (Fig. 1).

References

... H. L. Hammacher, L. Rosmann and K. Ahrén, *Acta endocr.* (Kbh.) 1974, 77 737–752.
... L. *Ann. N.Y. Acad. Sci.* 1970, 180 416–423.
... G. *Naunyn-Schmiedebergs Arch. Pharmak.* 1971 268, 272–299.
... D. A., J. R. Hoare and G. H. Wilkinson, *Biochim. Biophys. Acta* (Amst.) 1971 227 413–...

D 28

...staglandin Formation Participates in the Control of Tone and Contractility in the ...s Sphincter Muscle

By Lars Gustafsson and Per Hedqvist *Department of Physiology Karolinska Institutet, Stockholm, Sweden*

...s tissue has the enzyme system necessary for prostaglandin (PG) formation (van Dorp *et al.* 1967), and PG release from iris tissue has been noted (Posner 1973). In a previous communication we have reported that exogenous PGs enhance contraction responses to ...nergic nerve stimulation whereas PG synthesis inhibitors substantially reduce tone and contraction responses in the sphincter muscle of the bovine iris (Gustafsson *et al.* 1973). In the present study we wish to report on the effects of the PG precursor eicosa-tetraenoic acid (TEA) on smooth muscle tone and contractility in the bovine iris sphincter muscle.

Strips of the sphincter muscle were mounted in an organ bath with Tyrode at 37 °C and ...ated with 5% CO_2 in O_2. Transmural stimuli (5–15 biphasic pulses, 3 Hz, 1 ms pulse duration, *supramaximal voltage) were delivered by Grass S88 stimulator through platinum* electrodes along the wall of the bath at 1 min intervals.

TEA 3 µg/ml enhanced the contraction responses to transmural stimulation in 4 of 5 expts. ...d at 6 µg/ml it potentiated the responses in 6 of 6 expts. (p < 0.01). The responses increased over a period of approximately 10 min and then remained elevated until the preparation was washed. With the higher dose an increase in tone of the preparation was often seen. Prior administration of the PG synthesis inhibitor indomethacin 1 5 µg/ml, abolished the effects of TEA on tone and concentration responses while leaving the enhancing effect of PGE totally unaffected (Fig. 1).

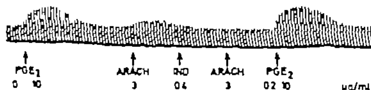


Fig. 1 Bovine iris sphincter muscle. Contraction responses to transmural stimulation 3 Hz, 15 pulses at 1 min intervals, 1 ms duration). Wash at dots. IND = indomethacin, ARACH = eicosatetraenoic acid, PGE_2 = prostaglandin E_2.

It is suggested that the stimulant effect of TEA is mediated by newly formed PG rathe than by TEA itself The results support the view that locally formed PGs may serve th function of controlling cholinergic neuroeffector transmission and muscular tone in th sphincter muscle of the bovine iris.

References

DORP D. A. VAN, G H JOUVENAZ and C. B. STRUIJK, *Biochim. Biophys. Acta* 1967 *137* 396–399.
GUSTAFSSON, L. P HEDQVIST and H LAGERCRANTZ, *Acta physiol. scand.* 1973 Suppl. 396, 106.
POSNER, J *Brit J Pharmacol* 1973 *49* 415–427

D 29

Total and Regional Oxygen Uptakes, Blood Flows and Arterial Pressures during Exercise with Smaller and Greater Muscle Groups

By N H SECHER, J P CLAUSEN, K. KLAUSEN, I NOER and J TRAP-JENSEN. *Laboratory for Theory of Gymnastics August Krogh Institute University of Copenhagen, and the Department of Clinical Physiology Frederiksberg Hospital Copenhagen, Denmark*

During dynamic exercise performed with the arms and the legs simultaneously the increase in oxygen uptake (ΔVO_2) is lower than ΔVO_2 for arm-exercise plus ΔVO_2 for leg exercise measured when the arms and the legs perform their shares of the total work load separately This could reflect either that ΔVO_2 in tissues not in exercising arms or legs makes up a considerable part of total ΔVO_2 during both types of exercise or that exercise with one muscle group may influence the oxygen supply to another active muscle group.

In the present study including three young healthy subjects, $\dot{V}O_2$, cardiac output and leg blood flow (indicator dilution methods) axillary and femoral arteriovenous oxygen differences ($AVDO_2$) and intraarterial blood pressures were measured during upright exercise on bicycle ergometer performed with and without concomitant arm-cranking as well as during arm-cranking alone. Arms and legs performed the same mechanical loads during all types of exercise, but arm-loads were on average 40% of leg loads. It was found that VO_2 in the exercising legs (leg blood flow times femoral $AVDO_2$) was practically the same whether the legs exercised alone or in combination with arm exercise (2.5 l O_2/min. vs 1 89 l O_2/min). Furthermore the difference between pulmonary $\dot{V}O_2$ during combined arm- and leg exercise and VO_2 in the active legs (3 46 l O_2/min – 1 89 l O_2/min. = 1.57 l O_2/min) was essentially the same as VO_2 during isolated arm-exercise (1.52 l O_2/min.). This suggests unimpaired O_2 supply to both arms and legs during combined exercise. Nonetheless leg-blood flow was reduced by 13% when arm-exercise was superimposed. Arterial mean blood pressure was unchanged or slightly increased (113 mm Hg vs 117 mm Hg) during combined exercise as compaired to leg exercise alone. Thus addition of arm-exercise caused an increased vascular resistance in the exercising legs. The main reason for this is probably an increased sympathetic vasoconstriction in the skin in proportion to the increase in relative work load HR increased from 174 to 192 beats per min. when arm-exercise was added to leg exercise.

D 30

...ical Magnification Factor in the Foveal Region of Vervet Monkey

By C. Gold, Institute of Neurophysiology, University of Copenhagen, Denmark

...quadrant of the retina projects to striate area on the occipital lobe, the projection ...described by the magnification factor M mm/degree i.e. the distance in mm on cortex ...sponding to 1° of visual field. M falls with increasing angle of visual field, is constant ...a semicircle and describes a regular projection from center of fovea to the border ...isual field (Daniel and Whitteridge 1961). Since later findings (Dow and Gouras 1973) ...d indicate that center of fovea is represented some distance from the border of striate ...ex, we examined the projection of fovea, 0°–4° by recording summated potentials ...ed at known locations on cortex by monochromatic small-spot flashes at known loca-...s on retina. M was calculated for all neighbouring pairs of positions and related to ...is of semicircle and angle of meridian in visual field.

...tween semicircles 0.4° and 0.9° M was the same (P > 0.5), i.e. constant along semi-...les also in the foveal area. 1/M as function of distance θ° from center of fovea gave the ...ession line 1/M = 0.065 + 0.074 θ up to 4° from fovea (N = 273, $S_{y,x}$ = 0.061, ME_b = ...), r = 0.72, P < 0.001). With 1/M = a + bθ the area on cortex is A = (π/b²) [ln(1 + bθ/a) − ...a, bθ)] mm² (0 ... 10°). For θ = 0.85° A = 106 mm² compared with 113 mm² calculated ...n corresponding coordinates on cortex.

...his model shows that retina also in the foveal area is mapped regularly on cortex. Thus ...center of fovea is represented at the border of striate cortex, in this monkey about ...nm posterior to the end of lunate sulcus and 3 mm above the border of occipital sulcus, ...agreement with the extent of striate cortex on histological preparations.

...rom Cowey and Rolls (1974) data on the cortical magnification factor in man, the ...ation 1/M = 0.073 + 0.053θ is valid up to 15° (N = 7, S_y = 0.018, ME_b = 0.0014, r = 0.998, ...0.001). Thus the magnification factor of monkey is similar to that of man at the ...ter of fovea, but it falls more steeply with increasing visual angle in monkey than in man ...to the smaller total visual area.

References

...wey, A. and Rolls, E. T.: Exp. Brain Res. 1974, 21, 447–454.
...niel, P. M. and Whitteridge, D.: J. Physiol. 1961, 159, 203–221.
...w, B. M. and Gouras, P.: J. Neurophysiol. 1973, 36, 80–100.

D 31

...yperpolarization of the Cell Membrane in Rat Liver after Partial Hepatectomy

By M. Palombino and M. Folke, Institute of Medical Physiology, Dept. A, University of Copenhagen, Juliane Mariesvej 28, DK-2100 Copenhagen, Denmark

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It is suggested that the stimulant effect of TEA is mediated by newly formed PG rather than by TEA itself The results support the view that locally formed PGs may serve the function of controlling cholinergic neuroeffector transmission and muscular tone in the sphincter muscle of the bovine iris.

References

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D 29

Total and Regional Oxygen Uptakes, Blood Flows and Arterial Pressures during Exercise with Smaller and Greater Muscle Groups

By N H SECHER, J P CLAUSEN K. KLAUSEN I NOER and J TRAP-JENSEN. *Laboratory for Theory of Gymnastics August Krogh Institute University of Copenhagen, and the Department of Clinical Physiology Frederiksberg Hospital, Copenhagen Denmark*

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In the present study including three young healthy subjects, VO_2, cardiac output and leg blood flow (indicator dilution methods) axillary and femoral arteriovenous oxygen differences ($AVDO_2$) and intraarterial blood pressures were measured during upright exercise on bicycle ergometer performed with and without concomitant arm-cranking as well as during arm-cranking alone. Arms and legs performed the same mechanical loads during all types of exercise, but arm loads were on average 40% of leg loads. It was found that VO_2 in the exercising legs (leg blood flow times femoral $AVDO_2$) was practically the same whether the legs exercised alone or in combination with arm exercise (2.5 l O_2/min. vs. 1 89 l O_2/min). Furthermore the difference between pulmonary VO_2 during combined arm- and leg exercise and VO in the active legs (3 46 l O_2/min.–1 89 l O_2/min. = 1 57 l O_2/min) was essentially the same as VO during isolated arm-exercise (1.52 l O_2/min.). This suggests unimpaired O supply to both arms and legs during combined exercise. Nonetheless leg-blood flow was reduced by 13% when arm-exercise was superimposed. Arterial mean blood pressure was unchanged or slightly increased (113 mm Hg vs 117 mm Hg) during combined exercise as compaired to leg exercise alone. Thus addition of arm-exercise caused an increased vascular resistance in the exercising legs. The main reason for this is probably an increased sympathetic vasoconstriction in the skin in proportion to the increase in relative work load HR increased from 174 to 192 beats per min when arm-exercise was added to leg exercise.

D 30

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References

...EY A and ROLLS, E T Exp Brain Res 1974 21 447–454.
...EL, P M and WHITTERIDGE, D J Physiol 1961 159 203–221
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D 31

...yperpolarization of the Cell Membrane in Rat Liver after Partial Hepatectomy

By M. PALOHEIMO and M. FOLKE, Institute of Medical Physiology Dept A, University of Copenhagen, Juliane Maries vej 28, DK 2100 Copenhagen, Denmark

...merous reports in the literature indicate that rat liver has a remarkable capacity for ...generation or compensatory hyperplasia. After a two thirds hepatectomy increased DNA

synthesis, preparatory to cell division can be detected at 12 h, mitosis at 24 hours, and by 48 h the weight of the liver remnant has doubled (*e g* Bucher *et al* 1969).

In the present study intracellular potentials were recorded *in vivo* from rat livers before and at various times after two thirds hepatectomy in order to see, whether changed membrane properties in the rapidly proliferating new cells would be reflected in such potentials. The recordings were made on fed rats weighing 195–250 g.

The potentials were in the normal preoperative range (40–44 mV) until 2–3 h after the resection, at which time an increase to 49–55 mV was seen Apart from a transient reduction at 5–7 h this hyperpolarization was maintained for approximately 24 h. During the rest of the regenerative phase the membrane potentials were normal

Thus, the time course of hyperpolarization does not suggest a relationship of membrane potentials to cell proliferation but rather to earlier events in the liver remnant.

The hepatic glycogen content is known to fall sharply after a two thirds hepatectomy reaching very low levels at 3–4 h (Simek *et al* 1968). The demand for blood glucose after this time must be met by gluconeogenesis. It has recently been shown that hyperpolarization occurs in isolated perfused rat livers upon stimulation of gluconeogenesis with glucagon (Friedmann and Dambach 1973) and with various gluconeogenic substrates (Dambach and Friedmann 1974).

These reports together with our own observations raise the possibility that hyperpolarization after partial hepatectomy is associated with intensified gluconeogenesis during the early adaptation of the liver remnant to unchanged demands from the rest of the organism Support for this hypothesis was obtained from additional experiments on rats fasted for 48 hours. In such rats hyperpolarization occurred as early as 10–20 minutes after the resection.

References

BUCHER, N L R., T R. SCHROCK and F L. MOOLTEN, *Hopkins Med. J* 1969 *125* 250–257
DAMBACH G and FRIEDMANN, *Biochim. Biophys. Act* 1974 *367* 366–370.
FRIEDMANN, N and G DAMBACH, *Biochim. Biophys Act* 1973 *307* 399–403
SIMEK J F F RUBIN and I LIEBERMAN *Biochem. Biophys Res Comm.* 1968. *30* 571 575.

D 32

Phase Dependent Reflex Reversal during Swimming in the Spinal Dogfish

By S. GRILLNER, S. ROSSIGNOL and P WALLÉN *Department of Physiology University of Göteborg Sweden*

Dogfishes can perform continuous swimming movements of the body even after a transection of the spinal cord (Gray and Sand 1946) This study is concerned with the response to a short lasting stimulation of the tail fin applied in different phases of the swimming movement.

Twelve dogfishes (Squaluas acanthias) spinalized 4 to 8 segments below the foramen magnum were placed in a tank and fixed by the snout (see Grillner 1974) Bipolar EMG recordings were obtained from pairs of copper wires (100 μm) implanted in the red lateral

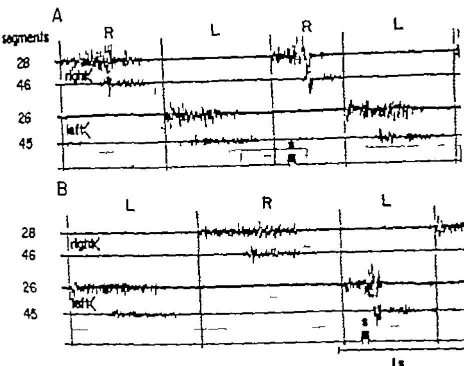


Fig. 1. Phase dependence of reflex responses to tail fin stimulation. The EMG from muscle segments 28 and 46 on the right and 26 and 45 on the left were recorded on a Mingograph ink writer. These levels correspond roughly to the first and second dorsal fins. Note the delay of activation from rostral to caudal segments and the strict alternation between the two sides. The dotted lines divide the right (R) and left (L) half cycles of swimming. In both sets *S* represents a train of 60 ms (18 pulses of 1 ms) at 2.5 mA. Note that the stimulating electrodes were placed subcutaneously on the right and the left side at the "root" of the tail fin and that the insulation on the copper wires has been removed for 3 mm on each electrode. One second calibration is given below B.

A. The stimulation occurs 110 ms after the onset of the EMG in the 2nd right half cycle. A large amplitude response follows in both segments on the right side only. The duration of the EMG burst is shorter and so is the corresponding half cycle. The following left half cycle is identical to the preceding one.
B. The same stimulation given a few seconds after the previous one but occurring 180 ms after the onset of EMG in the 2nd left half cycle. The pattern of response is the reversed image of that in A.

muscles. One such wire was inserted on each side of the root of the tail fin for stimulation. The stimulus could be delivered in any phase of the cycle through a variable delay unit triggered by the EMG activity in one channel. The fishes were filmed with a 16 mm camera synchronized with the EMG.

Fig. 1A shows the rhythmic alternating EMG activity on the two sides of the body. A short train of stimuli applied during the EMG activity on the right side gave a strong increase of the activity on this side but no contralateral effects. With an identical stimulation during EMG activity on the left side the pattern was reversed (Fig. 1B). Note that in both cases, the EMG bursts are shorter than the preceding locomotor bursts but of much higher amplitude resulting in a short but powerful stroke. Thus the same stimulus activates one side or the other in synergy with the phase of locomotion.

These results differ from those obtained with unilateral stimulation (Lissmann 1946), which only induces a withdrawal" to the contralateral side. With the present stimulus the reflex effect depends entirely on the swimming phase. Similar observations have been made in chronic spinal cats during walking (Forssberg *et al* 1975). Such reflex reversals could be explained by activation of alternate spinal pathways, phasically opened and closed by the central generator(s) for locomotion (see Grillner 1975).

References

Forssberg, H S. Grillner and S. Rossignol, *Brain Res.* 1975 *85* 103–107
Gray J and A. Sand, *J exp Biol.* 1936. *13* 200–209
Grillner, S *Exp Brain Res* 1974 *20* 459–470.
Grillner, S., *Physiol Rev* 1975 (in press).
Lissmann, H W *J xp. Biol.* 1946. *23* 143–161

D 33

Deterioration of Drug Biotransformation in Rat Liver by a Fungicide Dichlone

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The microsomal biotransformation system metabolizes a wide variety of both exogenous and endogenous substrates to form more polar excretable end products. This occurs often in two subsequent phases, oxidation followed by conjugation, in mammals generally with glucuronic acid. The enzymes catalyzing the two enzymic steps are tightly bound to the microsomal cell fraction. Rather little is known about the topochemistry and possible linkage of the two enzyme machineries in the membrane structure. To gain further information of the above facts, the fungicide dichlone (2,3-dichloro-1 4-naphtoquinone) was administered intraperitoneally to rats at dose levels of 2, 5 and 10 mg/kg b wt. This fungicide has been suggested to be bound to the sulphydryl groups of the membranes, and thereby interfere with the function of *e g* erythrocytes (Siikka *et al* 1974).

The 10 mg/kg b.wt. dose of dichlone was already observed to be very lethal to rats, only one from eight rats did survive 24 h after the dosage. The administration of dichlone to rats caused a profound decline in the activities of the overall drug hydroxylation reactions, both hydroxylation of 3 4-benzpyrene and O-demethylation of p-nitroanisole being decreased to about 50 per cent of the control with a dose of 5 mg/kg b wt. With this dose level NADPH cytochrome *c* reductase activity was decreased by 40 per cent and the content of cytochrome P-450 in liver microsomes was declined to about 50 per cent. Also the microsomal enzyme acting on epoxides, epoxide hydrase, was inhibited similarly to the monooxygenase system.

UDPglucuronosyltransferase, the enzyme responsible for the conjugation of the (preformed) hydroxylated products has been suggested to have a deep location in the microsomal membrane, perhaps in its inner compartment (Vainio 1973) Its activity was not detectably altered by dichlone administration to rats, not even in microsomes treated *in vitro* with digitonin and trypsin to detect also the latent transferase activity

The above results indicate that the fungicide dichlone is rather toxic for rats, the lethal being about 10 mg/kg b.wt. Of the microsomal biotransformation reactions, monooxygenase and epoxide hydrase were readily affected by dichlone administration whereas glucuronosyltransferase was more resistant towards dichlone treatment. Thus the present data support our earlier assumptions on the more protected microenvironment of glucuronosyltransferase in the microsomal membrane than the mono-oxygenase system (Vainio 1973).

References

A, H. C., Schwartzel, E. H. Saxena, J. and Zweig, G., *Abstract Book, The Third International Congress of Pesticide Chemistry.* Helsinki 1974. 228.
Vainio, H. *On the Topology and Synthesis of Drug-Metabolizing Enzymes in Hepatic Endoplasmic Reticulum.* Thesis, Turku, 1973. Pp. 1–46.

D 34

Hepatic Biotransformation of Xenobiotics in the Gunn Rat

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The Gunn strain of the conventional Wistar rat is known to exhibit congenital non-hemolytic hyperbilirubinemia due to an almost complete lack of the activity of the enzyme UDPglucuronosyltransferase towards bilirubin (Carbone and Grodsky 1957). On the other hand, Gunn rat is able to glucuronidate some exogenous compounds, e.g. p-nitrophenol practically normally (Dutton, 1971).

In the present study we investigated glucuronyl, glucosyl, glutathione and glycine conjugations and epoxide hydration in livers of Wistar and Gunn rats to clarify the possible compensatory mechanisms of the drug biotransformation and to further study the nature of the glucuronidation defect in the Gunn rat.

The activities of bilirubin UDPglucuronosyl- and UDPglucosyltransferase were in hepatic microsomes of homozygous Gunn rats less than 10% of those in Wistar rats. In heterozygous Gunn rats the activities were 60–70% smaller than in Wistar rats. Treatment of microsomes *in vitro* with digitonin, trypsin or phospholipase C enhanced the measurable activities of UDPglucuronosyl- and UDPglucosyltransferase with equal relative magnitude in all three groups of animals. When 4-methylumbelliferone was used as the aglycone substrate of UDPglucuronosyltransferase the activity of the enzyme was in non-treated microsomes of homozygous and heterozygous Gunn rats over 60% of that in Wistars. The pretreatment of the microsomes activated also the enzyme of the Gunn rats but did not bring it to the level obtained in pretreated microsomes of Wistar rats.

The activities of hepatic microsomal epoxide hydrase and the supernatant enzyme glutathione S-epoxide transferase (both determined with styrene oxide as substrate) did not differ significantly in Gunn rats from the values of Wistar rats. The activity of glycine N-acyltransferase of isolated mitochondria was, however, in Gunn homozygotes significantly (about 65%) higher than in Wistar rats. Also in heterozygotes the activity appeared to be slightly higher (about 35%) than that in Wistar rats.

The reciprocal velocity (Lineweaver–Burk) plots of hepatic UDPglucuronosyltransferase with varied UDPglucuronic concentrations bent downwards at low UDPglucuronic acid concentrations similarly in microsomes of Wistar and homozygous and heterozygous Gunn rats when determined with ^{14}C labeled p-nitrophenol as substrate. Thus the kinetics of UDP glucuronosyltransferase appeared to be similar in normal and jaundiced rats in spite of the lower activity of the latter ones.

Our results indicate that the hyperbilirubinemic Gunn rats are as deficient in UDP glucosyltransferase as in UDPglucuronosyltransferase activity towards bilirubin. Other drug detoxifying enzymes studied were, however at normal or even elevated level in Gunn rats. The kinetic and activation studies supported the concept of normal microenvironment of microsomal UDPglucuronosyltransferase in the Gunn rat.

References

CARBONE, J V and G M GRODSKY *Proc Soc exp. Biol* (N.Y) *1957 94* 461–463.
DUTTON G J *Handbook f Experimental Pharmacology 1971* XXVIII. Springer Berlin-Heidelberg-New Yo k, pp 378–400.

D 35

Effect of Beta-receptor Blockade on Heart Rate, Hepatic Blood Flow and Circulating Noradrenaline during Exercise in Man

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Beta-receptor blocking agents cause a reduction in heart rate, in cardiac output and in arterial blood pressure both at rest and during exercise. The impaired cardiac response to the sympathetic stimulation during exercise could be anticipated to cause a compensatory increased peripheral vasoconstriction.

The present investigation was undertaken to study the effect of long-term beta-receptor blockade on heart rate (HR), on peripheral vasoconstriction as judged by changes in the splanchnic-hepatic blood flow (SBF) and on arterial plasma noradrenaline concentration (NA). HR SBF and NA were measured at rest supine and during 20 min of upright submaximal exercise on a bicycle ergometer in six male subjects with mild essential hypertension (mean age 35 years) before and after 4 to 8 months daily administration of 160 to 320 mg propranolol perorally The subjects exercised at the same absolute work loads during both studies. Catheters were placed in the main right hepatic vein and in the common iliac artery ECG was continuously recorded, SBF was measured using constant infusion of indocyanine-green. The NA concentration was determined using a precise and sensitive double isotope derivative technique.

During exercise the reduction in SBF was accompanied by an increase in NA from rest to exercise (Δ NA) and SBF expressed as per cent of the value at rest (SBF%) was closely correlated to Δ NA ($p < 0.01$). The increase in HR over the value at rest (Δ HR) was also

...ely correlated to SBF% (Clausen and Trap-Jensen, 1974 Rowell, 1974) as well as to ... (Christensen and Brandsborg, 1973).

...ng beta-receptor blockade the relation between SBF% and Δ NA remained un...d although SBF in mean was 33 per cent lower and Δ NA was increased by a factor ...at the same oxygen uptake. In contrast the relation between Δ HR and Δ NA showed ...lel displacement to the left by about 50 beats per min.

...present observations showed that during exercise different parameters (HR, SBF ...A) reflecting the degree of activation of the sympathetic nervous system changes ...portion to each other They showed in addition that the suppression of the cardiac ...se to exercise caused by beta-receptor blockade leads to a compensatory alfa-receptor ...tion of resistance vessels in non-working tissues with a concomitant proportional in... in circulating NA.

References

...sen, N. J. and Brandsborg, O., *Europ. J. clin. Invest.* 1973, 3, 299–306.
...n, J. P. and Trap-Jensen, J. *J. appl. Physiol.* 1974, 37 716–719
...l, L. B. *Physiol. Rev.* 1974, 54 75–159

D 36

...ing at Supramaximal Ventilations during Rebreathing and Steady State Exercise

by Jakob Mertz, Poul-Erik Paulev and S...end Stenvang Pedersen, *Institute of Medical Physiology B, University of Copenhagen, Denmark*

... is a linear relation between pulmonary ventilation (V_E) and tidal volume (V) ...g steady state CO_2 breathing at rest and during moderate exercise, when breathing ...p to a V equal to approximately half the vital capacity (Hey *et al.* 1966). With ...onal increases in V̇ (up to 80 L (BTPS)/min.) Hey *et al.* found no further increase in ...Similar results were obtained in initially eupnoeic cats (von Euler Herrero and Wexler ...). During rebreathing in man, the frequency (f) was found rather constant at ventila... up to 30 L/min, range 1 and with further increments in V̇ a concomitant, almost ...r rise in f was observed, range 2 (Clark and von Euler 1972).

...ntilation was measured breath-by-breath with a system described earlier (Paulev 1971). ...end-tidal gas tensions were obtained with Beckman instruments.

...our subjects were examined during rebreathing and cycling at different work rates. ...these experiments a range 1 was not observed, but at supramaximal ventilation, a fall ... and rise in f was obtained (Fig. 1). At the highest steady state work rate (1800 ...m/min) ...e reached ...entilations up to 200 L (BTPS/min) (e.g. actually the ...a erage, ...ratory ...olume-rates per breath) at end-tidal P_{CO_2} (P_{ETCO_2}) values just above 100 mm .... These ...entilations exceed the maximal voluntary ...entilation measured in 15 seconds ...(V_{max}).

At the supramaximal ventilations, the V was almost constant at approximately 170 L ...(BTPS)/min, while the V decreased 1 1.5 L (BTPS) with a corresponding increase in f to

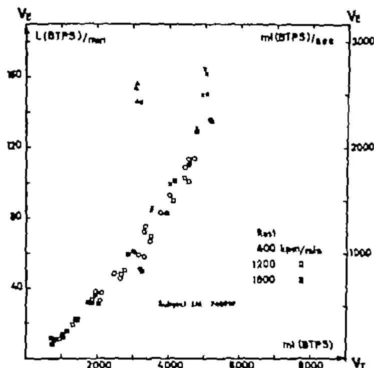


Fig. 1 The expiratory olume rate is shown in relation to the expiratory tidal olume, V_T, for each breath i one subject.

45–50 *breaths/min in all 4* subjects. The explanation of the present phenomenon (*i.e* the *fall in* V_E and the rise in f at supra maximal ventilations) may include one of the following possibilities or their combination

First, at high levels of inspiratory flow an intercostal muscle-phrenic" reflex derived from the intercostal muscle spindles in response to chest wall distortion cause an inhibition of phrenic activity in cats and dogs (Remmers 1970)

Secondly we observed a rise in rectal temperature of approximately 0.5 C from rest to work at 1800 kpm/min. Part of the "shift to the left" in the V_E vs. V_T plot, which was observed at the two highest work rates (Fig. 1) may be explained by a temperature effect (Vejby-Christensen and Strange Petersen 1973). Hey *et al* (1966) found, that raised temperature is the only respiratory stimulus, which affects the V_E–V_T relation. Since the V_T was falling more and more (Δ in Fig. 1) with increasing P_{ETCO_2} during the last 45–60 seconds of the exercise—rebreathing combination a P_{CO_2} stimulus must also be involved.

References

Clark, F J and Euler, C. von *J Physiol.* 1972. *222* 67 795

Euler, C. von, Herrero, F and Wexler, I *Resp Physiol* 1970. *10* 93–108

Hey E. N., Lloyd, B. B., Cunningham, D J C Jukes, M G M and Bolton, D P G *Resp. Physiol* 1966. *1* 193–205

Paulev P E. *J appl. Physiol.* 1971 *30* 165–172.

Remmers, J E., *Resp. Physiol.* 1970. *10* 358–383

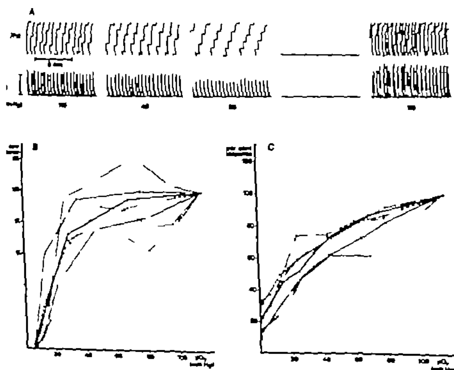
Vejby-Christensen H. and Strange Petersen, E., *Resp Physiol.* 1973 *19* 322 332.

D 37

...en Sensitivity of Spontaneous and Induced Contractions in Venous Smooth Muscle

by PER HELLSTRAND, *Department of Physiology and Biophysics, University of Lund, Sweden*

...contractile response of arterial smooth muscle to adrenergic stimulation depends on ...(Detar and Bohr 1968, Pittman and Duling 1973). It is of interest, however to investi... also the effect of low pO_2 on vessels showing myogenic tone, since variation of such ...is important for regulating flow in the microcirculation (*e.g.* Mellander and Johans... 1968). Flow resistance of an isolated arterial segment has been shown to vary with pO ...ner Walker and Guyton 1964). For an understanding of the cellular mechanisms of ...ea dependence however direct study of the vascular smooth muscle is needed. In the ...nt work the influence of pO_2 on spontaneous and induced contractions of the isolated ...portal vein has been determined.



...1. (A) Patterns of spontaneous activity appearing in portal vein at 5 different levels of pO_2 applied ...order (from left to right. Lower record: tension. Upper record: time-integral of tension (arbitrary units, ...min interval). Note increased activity as oxygen ... readmitted after hypoxia. (B) Integrated spontaneous ...ivity at successively lower pO... 7 preparations. Each point represents integral over 15 min as ... per...ntage of control response for same muscle at 113 mm Hg. (C) Contracture tension on 2 mm K... depol...ization as function of pO_2 6 experiments. Successively lower pO_2 applied ... th 30 min between stimula...ns. Control response for each muscle set at 100 ...

6, 110

Longitudinally opened veins (wall thickness 100–150 μ) were mounted in a bath. The patterns of spontaneous activity appearing as pO_2 was lowered in steps from 115 mm Hg to 1 mm Hg are exemplified in Fig. 1A. At reduced pO both duration and amplitude of each phasic contraction were diminished. After the hypoxic period there was a transient increase of activity lasting about 20 min. In Fig. 1B the time integral of tension is plotted against pO_2. A steep dependence is noted in the range below 50 mm Hg. The sustained tension developed on K -depolarization is also oxygen-dependent, as shown in Fig. 1C. However at 1 mm Hg the muscles were still capable of developing 23.2 ± 3.3% (mean $\pm$S.E., n = 6) of control tension. Maximal stimulation with noradrenaline at 1 mm Hg gave an integrated tension of 26.9 ± 1.7% of control response.

This study has shown a marked oxygen dependence of the myogenic activity of a vascular smooth muscle preparation in ranges of pO occurring physiologically. The effect does not seem to be entirely due to depletion of energy supplies needed for contraction, since hypoxia appears to cause also an inhibition of membrane events triggering the spontaneous contractions.

References

CARRIER, O JR., J. R. WALKER and A. C. GUYTON, *Amer. J. Physiol.* 1964, 206, 951–954.
DETAR, R. and D. F. BOHR, *Amer. J. Physiol.* 1968, 214, 41–44.
MELLANDER, S. and B. JOHANSSON, *Pharmacol. Rev.* 1968, 20, 117–196.
PITTMAN, R. N. and B. R. DULING, *Microvasc. Res.* 1973, 6, 202–11.

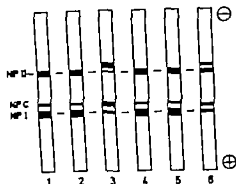
D 38

Heat Inactivation of Bovine Neurophysins

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The neurophysins are low molecular weight proteins found in the neural lobe of the pituitary gland. They are characterized by their ability to bind the hormones vasopressin and oxytocin. In the bovine neurohypophysis three such proteins have been isolated and named neurophysin I, C and II in order of decreasing electrophoretic mobility at pH 8.1 (Hollenberg and Hope 1968, Rauch *et al.* 1969).

In the present study extracts of fresh bovine neural lobes were subjected to polyacrylamide gel electrophoresis. The electrophoretic mobility of the neurophysins was identical to the mobility of neurophysins purified from preparations of acetone-dried bovine posterior pituitaries (Fig. 1). If however the extract of fresh neural lobe tissue was heated (100 C) for 20 min a change in the mobility of neurophysin I and II on the gels was found. This was not observed when solutions of purified neurophysins were treated in the same way. Since neurophysins are rich in disulfide bonds it was possible that the fresh extracts contained reducing agents and that heat treatment accelerated a reduction of the neurophysins. To test this hypothesis fresh extracts were heated in the presence of 5 mM potassium ferricyanide. The presence of this oxidant in the extract completely inhibited the degradation of



[...]lyacrylamid-gel electrophoresis of bovine [...]ns. 1) neural lobe extract, 2) purified [...]ns, 3) heat treated neural lobe extract, [...]ated purified neurophysins, 5) neural lobe [...]t treated in the presence of $K_4Fe(CN)_6$, [...] neurophysins heat treated in the presence [...]ione.

[...]ysin I and II. When a solution of purified neurophysins was heat treated in the [...] of 2 mM glutathione a change in the electrophoretic mobility could be demon[...]. It is concluded that extracts of fresh neural lobe tissue contain reducing substances [...] the effect of heating of the extracts could be reduction of the disulfide bonds of [...]ophysins or mixed disulfide formation. Finally it could be demonstrated by affinity [...]tography that the heat induced changes of the neurophysins had abolished their [...]o bind [...]asopressin.

References

[...]ING, M. D. and D. B. HOPE. Biochem. J. 1968, 106, 557–564.
[...]R. M. D. HOLLENBERG and D. B. HOPE. Biochem. J. 1969, 113, 473–479.

D 39

[...]lar Transport of Proteins from Blood to Brain during Acute Hypertension

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[...]enously injected horseradish peroxidase (HRP) has been demonstrated to cross, by [...]lar transport, the walls of very few and short segments of cerebral arterioles, capillaries [...]ules in mice and rats (Westergaard 1974a, Nørtved and Westergaard 1974).

[...]ring acute hypertension (HT) evoked by metaraminol bitartrate (Aramine) the blood-[...] permeability to Evans Blue and to HRP is increased (Olsson and Hossmann 1970, [...]sson 1974).

[...]e purpose of this study was to see if acute HT increases the modest vesicular transport [...]rved in cerebral vessels and to identify the routes taken by the protein tracer.

[...]e arterial blood pressure (BP) was continuously recorded from the femoral artery of [...] rats anaesthetized with sodium-pentobarbitone. During the first 40 min the BP was [...]-120 mmHg. Aramine was infused i.[...] at 0.07 mg per kg per min immediately resulting [...] well-defined BP of 170–190 mmHg. 10 min later 100 mg of HRP (Sigma, type II) [...] slowly infused and circulated for 15 min followed by cardial perfusion with aldehydes.

The brain was removed, cut into slices and incubated. Some of the tissue sections were cleared Other samples were processed for electron microscopy

The cleared sections showed a very marked increase of reaction product as compared with controls not receiving Aramine, both in the walls of many larger vessels, in the neuropil and in the subarachnoid space.

Electron microscopic examination showed reaction product in the walls of many arterioles and venules, in the basement membranes under their endotheliae and in surrounding smooth muscle cells, in the perivascular spaces and in the intercellular spaces of the neuropil nearby

Higher magnification showed that HRP did not pass the first luminal tight junction. The intercellular spaces were filled retrogradely with HRP from the subendothelial basement membrane up to the first abluminal tight junction. The cytoplasma of endothelial cells contained several vesicles filled with HRP No endothelial cell damage was demonstrated.

In conclusion, acute HT induced by Aramine strongly increased the normally occurring vesicular transport of HRP through the walls of cerebral vessels while the tight junctions effectively prevented any escape of HRP between endothelial cells. However beside any effect of HT upon the vesicular transport it cannot be ruled out that Aramine itself might induce vesicular transport since it has recently been shown that serotonin and norepinephrine with molecular structures resembling that of Aramine both induce vesicular transport (Westergaard 1974 *b*)

References

JOHANSSON, B *Blood Brain Barrier Dysfunction in Acute Arterial Hypertension* Gotab, Kungälv Sweden 1974.

NORTVED, J and E. WESTERGAARD *Anat Rec* 1974. *178* 428.

OLSSON, Y and K. A. HOSSMANN *Acta Neuropath.* (Berl) 1970. *16*, 117

WESTERGAARD, E., in *Pathology of Cerebral Microcirculation.* (ed. J Cervós-Navarro). 1974 *a*. P 18.

WESTERGAARD, E., *Ultrastruct Res.* 1974 *b* In press.

D 41

An Electrode for Micropuncture Measurements of P_{CO_2}

By BERTIL KARLMARK and MORGAN SOHTELL *Department of Physiology and Medical Biophysics Biomedical Center University of Uppsala Sweden*

When studying metabolic and transport processes in living tissue it is of great importance to take into account the tissue handling of acid and basis. Regarding the bicarbonate system, very little is known about its regulation at the cellular level When carbon dioxide is produced, it will give rise to bicarbonate and hydrogen ions in relation to other intra- as well as extracellular buffers. The transport of carbon dioxide across a barrier thus also includes the transport of bicarbonate and hydrogen ions. Micropuncture investigations of transport processes for the components in the carbon dioxide system at a cellular level has, for technical reasons been undertaken in an indirect manner requiring knowledge of the actual in vivo pH In such experiments nano-liter samples are taken for equilibration

nown carbon dioxide tensions and the actual values of bicarbonate and P_{CO_2} can be ated from the buffer curve obtained. The evaluation of the bicarbonate transport nism are furthermore mainly based upon the hypothesis, that the carbon dioxide s in the extracellular space and in the cells of the proximal tubule of the kidney entical ith that in the arterial blood. However an increasing amount of information estioning this identity These findings might mean that the bicarbonate buffer system is a chemical equilibrium and therefore the variables have to be individually measured. electrode for the direct measurements of P_{CO_2} in tissue was constructed and was d for the use in proximal tubular micropuncture experiments on the rat kidney It ts of an Sb/Sb_2O_3–Ag/AgCl electrode system for measurements of the pH in a ardized bicarbonate solution. This solution together with the electrodes are enclosed tip of the cannula with a tip diameter of about 6 µm. The very tip is closed with nbrane which is diffusible for carbon dioxide. The pH sensitive surface of the antimony ode is placed in the bicarbonate solution just near the membrane. The response time e electrode is less than 3 min and the mean sensitivity is more than 90% of the ex- d value.

D 42

nation of the Noradrenaline Content in Sympathetic Nerve Terminal

By H. Lagercrantz, G Fried and T Hökfelt *Departments of Physiology and Histology Karolinska Institute, Stockholm, S eden*

noradrenaline (NA) content in each sympathetic nerve terminal vesicle is of interest he discussion of quantal release from nerve terminals. However direct determinations he NA content in nerve terminal vesicles have not been done since pure preparations ese particles are not yet available. The aim of the present study is to describe possible ect methods to estimate the NA content in both large dense-cored and small dense- d vesicles occurring in the nerve arikosities.

arge nerve vesicles were isolated (80–90% purity) from bovine splenic nerve axons by rential and density gradient centrifugation (see Lagercrantz, Kirksey and Klein 1974). estimate the NA content in the terminal vesicles the ratio between NA and dopamine ydroxylase was determined in different segments of the axons and in crude large vesicle parations from the spleen. Furthermore the NA loss during the *post-mortem* delay had e considered e g. the time interval between the animals were killed until the nerves could chilled on ice. Therefore the nerves and spleens were taken at different *post-mortem* ays and the NA/protein ratios in the different preparations were plotted to the mo- nt the animals were killed.

Estimation of the NA content in the small nerve esicles was carried out on preparations m seminal ducts from normal and castrated male rats. By castration of the rats the muscle er atrofied after about 3 weeks while the nerve arikosities were still intact. The small ve vesicles were isolated by differential and density gradient centrifugation essentially described by Bisby and Fillenz (1971).

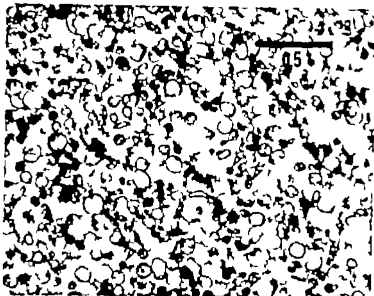


Fig. 1 NA storage particles isolated from seminal ducts of castrated rats by differential centrifugation (10 000 g for 30 min) followed by allowing the obtained supernatant directly on density gradients (0.25–0.8 M sucrose) which then were ultracentrifuged. The fraction with the highest NA and dopamine β-hydroxylase activity (about 0.45 M) was diluted to isotonicity and mixed with glutaraldehyde and then centrifuged at a high speed. The pellet was then postfixed and embedded.

The NA/protein ratio was estimated to be 65–85 μg/mg protein in the large nerve vesicles in the bovine spleen corresponding to 2–4 10^{-4} ng NA/vesicle. The NA/protein ratio in the small nerve vesicle preparations of the rat vas deference from normal rats was found to be 892 ± 50 (S.E., n 7) ng/mg protein and 2796 ± 705 (S.E.) n = 3) ng/mg from the castrated rats. The highest ratio was 4349 ng/mg.

A micrograph (Fig. 1) of the vesicle preparation from vas deferens of the castrated rats shows quite a high occurrence of small dense-cored vesicles indicating considerable lower NA content in these vesicles than earlier estimated By Dahlstrom, Häggendal and Hökfelt (1966). They estimated the NA content per vesicle to be 2–4 10^{-4} pg on the basis of analyses of the number of varicosities and NA content per unit tissue of rat vas deferens.

References

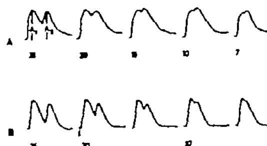
Bisby M A and M Fillenz, J Physiol 1971 215 163–179
Dahlström, A., J H ggendal and T Hökfelt A ta ph iol scand 1966. 67 289–294
Lagercrantz, H D F Kirksey and R L Klein, J Neurochem 1974 23 769–773.

D 42

Summation of Receptor Potentials in the Retina of the Fly *Calliphora* Studied by Very Short Double Flashes

By M Järvilehto and J Moring *Department of Physiology University of Oulu, Finland*

Some studies on receptor potential summation have shown that the second potential response amplitude can be either increased or depressed (mechanoreceptors, Gray and Sato 1953 stretch receptors, Ottoson, McReynolds and Shepherd 1969 light receptors, Duncan and Croghan 1973). These relations were studied in the visual receptor cells of the fly



1. Two potential series recorded from two different receptor cells (R_{1-6}). In (A) the second response amplitude (A_2) is increased and in (B) depressed by the ISI. Stimulus: 10 µs light flash. ISI interstimulus interval ms.

Dark-adapted retinal receptors were stimulated by very short (10 µs) double lig[ht flashes fro]m a stroboscope (Soc. AN–NA, Milano), programmed by a pulse generator. T[he ev]oked potential responses were recorded intracellularly from reticular cells by [gl]ass capillary microelectrodes. The cells were identified by a histological metho[d, i.e.] staining with Procion yellow and localization of the recording site by freez[ing] (Hardie and Zettler 1973).

The present results are based on 12 receptor cells. The summation of the two [re]sponses is not consistent. In six cases the second response amplitude is depressed, [in six] is increased (Fig. 1). If the interstimulus interval (ISI) is 100 ms or more, the ra[tio of the am]plitudes of the two responses (A_2/A_1) is about 1.0. By very short ISI's (7–15 ms) [it is] in some receptors more than 1.0 and in some less than 1.0.

Related to the high flicker fusion frequencies obtained in optomotor studies, [it is possi]ble to distinguish the response to the second stimulus from the potential comple[x at short] ISI's.

No correlation could be found between polarization sensitivity and A_2/A ra[tio]. The differences between the ratios in various cells could be explained on th[e basis of] similar adaptation mechanisms in the receptors.

References

[La]ng, G. and Cr[...]an, P. C.: *Biochemistry and Physiology of Visual Pigments* (ed. ...) Springer, New York, 1973.
[...]a, J. A. B. and Sato, M.: *J. Physiol.* 1953, *122*, 610–634.
[...]in, M. and Zettler, F.: *Z. Zellforsch.* 1973, *136*, 291–306.
[...]son, D., McReynolds, J. S. and Sherman, O. M.: *J. Neurophysiol.* 1969, *32*, 24–34.

D 44

Regulation of Cell Growth and Prolactin Production in Cultured Rat Pit[uitary ...]

By H. Tjernshaugen, E. Haug[1] and K. M. Gautvik. *Institute of [Bio]chemistry, University of Oslo, Norway, Institute of Physiology, Univers[ity of Oslo,] Norway and Hormone and Isotope Laboratory, Aker Hospital, Oslo, Nor[way]*

A deoxyinosine-activated 3′ and 5′-nucleotidase which is present in most tissues, has been suggested to be involved in the control of DNA/RNA synth[esis ...]

entiated cellular functions is insufficiently understood. Monolayer cultures of differenti-ated rat pituitary cells (GH_3) synthesize and secrete prolactin (PRL) into the culture medium (Tashjian and Hoyt 1972).

In spontaneously growing cells and in oestradiol treated cultures we have measured the intra and extracellular concentrations of PRL as well as the nucleotidase activity. The different phases of cell growth were monitored by measurements of total cell protein and incorporation of ^{3}H thymidine into DNA. The GH cells grow exponentially for about 8 days, after which they reach a plateau phase where cell growth and H-thymidine incorporation into DNA are greatly reduced. During exponential growth the nucleotidase activity was unchanged, in contrast to the spontaneous rate of extracellular PRL accumulation which increased about 3 times. At the early plateau phase there was a rapid decline in PRL production rate to near initial values. During this growth phase the nucleotidase activity was doubled and the rate of DNA synthesis reduced by 50 %. When the cells were cultured in the presence of 17 β-oestradiol (10⁻ M) the rate of PRL production increased more than 100% with no change in the nucleotidase activity. The incorporation of H thymidine into DNA was reduced by the oestradiol treatment.

These experiments show that the hormone production is most efficient when the cell is about to diminish the synthesis of macromolecules important for cell growth and division. Oestradiol treatment did not alter this pattern of basal hormone production, but it was able to stimulate PRL production at all different growth phases.

References

FRITZSON P. *Eur. J. Biochem.* 1972. 27. 38–4..

TASHJIAN A. H. Jr. and R. F. HOYT Jr. *Proceedings of the Symposium on Molecular Genetics and Developmental Biology* (ed. M. Sussman). Prentice-Hall, New Jersey 1972. Pp. 353–387.

D 45

Persistant Effects of Large Doses of Electrolytes in the Food of Lactating Rats on the Kidney Function of Their Offspring

By A. FREDRIKSSON and U. SÖDERBERG. *Neurophysiological Laboratory, Ulleråker Hospital, Uppsala, Sweden*

The significance of birth in developmental endocrinology has received increasing attention since the discovery that hormonal conditions at birth will determine maturation of endocrine regulatory systems with serious permanent consequences (*e.g.* Harris and Levine 1965). Physiological changes at birth (Dawes 1968) profoundly influence the endocrines of the newborn due to cessation of maternofetal correlations and placental action. In addition endocrinal consequences of new nutritional conditions exist (Jost and Picon 1970). This investigation was prompted by the finding of a reduced rate of elimination of radioiodine from offspring of rats treated with lithium during pregnancy and lactation (Söderberg 1973). Even if renal handling of electrolytes is impaired in hypothyroid rats (Michael *et al.* 1972) and lithium interferes with thyroid activity, the observed extrathyroidal effects might have been induced outside the pituitary-thyroid axis. Therefore, lithium and sodium chloride

nched diets were tested against a standard diet in pregnant and lactating rats. The off ing (n 207) were allowed to reach adult age, then studied for fate and distribution of iodine.

In contrast to lithium action on thyroid parameters, the effects on extrathyroidal iodine re found to depend on salt, similarly after equimolar amounts of lithium and sodium loride. Further the effects were much heavier in animals treated only during lactation, nas levels of radioiodine (4 h after a single dose) being 35 and 37% higher than in male d female controls respectively. There was also a significant 8% reduction in kidney eight. Serum levels paralleled iodine levels in muscle, liver kidney and testicular tissues t not in thyroid glands of the lithium rats. (Fagerberg, Fredriksson and Söderberg, to published.)

The finding that salt load of lactating rats influenced the off-spring with permanent pairment of renal function may be either an effect of transient endocrine changes in the others or a direct action on the newborns. Rats are very sensitive to salt excess, but it is open question whether or not other hormones than aldosterone are also involved (Levinky 1974). Assuming that some transient change in hormones responsible for electrolyte gulation is induced during a critical period of the development of the offspring, changes maturation of regulatory systems might take place in analogy with changes in pituitary-onadal and pituitary-thyroid system by other hormones. If dehydration, then, will cause milar effects as salt load, the present results have to be considered also in interpreting xperiments involving disturbances in suckling behaviour and maternal water intake.

References

Dawes, G. S. *Foetal and Neonatal physiology. A comparative study of the changes at birth.* Year Book Publ., Chicago, Ill. 1968.
Harris, G. W. and S. Levine, *J. Physiol.* 1965. *181.* 379–400.
Jost, A. and L. Picon, *Advanc. metabol. Disord.* 1970. *4.* 123–184.
Michell, U. F. R. L. Barenberg, R. Chavez, C. A. Vaamonde and S. Papper, *J. Clin. Invest.* 1972. *51.* 1405–1412.
Levinsky, N. G. *Advanc. metabol. Disord.* 1974. *7.* 37–71.
Söderberg, U. *Nord. psykiatr. Tidskr.* 1973. 27. 414–419.

D 46

Energy Metabolism in Different Human Skeletal Muscles during Voluntary Isometric Contraction

By G. Bolstad and A. Ersland, *Institute of Physiology, University of Bergen, Norway*

Studies on isolated hamster muscles at 2°C have suggested a higher energy turnover in m. biceps than in m. soleus at comparable tensions (Goldspinck *et al.* 1970). The present study was undertaken to clarify whether similar differences exist amongst different skeletal muscles in man.

The rate of temperature increase was measured during maximal voluntary isometric contraction (MVC) and circulatory arrest in m. soleus, m. sacrospinalis and m. biceps in 22 male students. Nyloninsulated 36-gauge copper/constantan thermo-elements were in-

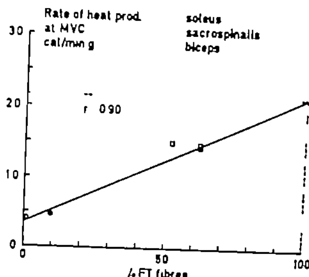


Fig. 1 Maximal rate of heat produc... different muscles related to percentag... tent of fast twitch (FT) fibres.

serted 5–6 cm into the muscle through a hypodermic needle. Provided no heat is lost f... the monitored region the local heat production in the muscle can be calculated as $\dot{Q}$ ... Δt q cal/min g, where $\Delta T/\Delta t$ is the temperature rise per minute and q is the specific h... of muscle estimated as 0.9 cal/g.

$\dot{Q}$ rose in all experiments with increasing force of contraction. The mean rate of h... production at MVC was 0.55 cal/min g (range 0.29–0.90, n = 11) in soleus, 0.66 cal/mi... (range 0.41–1.07 n = 6) in sacrospinalis and 1.48 cal/min g (range 1.30–1.71 n = 5) in ... ceps.

Muscle biopsies were taken from 14 muscles and fibre type distribution determi... histochemically by staining for ATPase at pH 9.4

The rate of heat production at MVC showed positive correlation to the area of f... twitch (FT) fibres given as percent of the cross-section area of the muscle (Fig. 1). The r... gression line is given by $\dot{Q} = 0.018$% FT + 0.35 r = 0.90.

Linear extrapolation suggests that a muscle composed exclusively of FT fibres wou... have a maximal heat production of 2.15 cal/min g, whereas a muscle containing slow twit... (ST) fibres only would have a maximum of 0.35 cal/min g.

Thus, according to this estimate, the maximal energy turnover in human FT fibres ... approximately six times that of ST fibres during voluntary isometric contraction.

Reference

GOLLNICK, G. R. E. LARSON and R. E. DAVIS, Z. ergl. Physiol. 1970 66 389–397

D 47

Metabolism of Iodine and Iodinated Thyroid Products in Amphibia

By P. ROSENKILDE. *Zoophysiological Laboratory A. University of Copenhagen, 15 Uni-versitetsparken Denmark*

A survey of thyroid hormone dynamics in adult amphibia is attempted. The most com-plete investigation was made on the common toad Bufo bufo. I 131 uptake, total iodine

centration and relative distribution of iodinated products in plasma, and biological half-life of I-125 labelled thyroxine. Further it was attempted to obtain isotopic equilibration of all iodine compartments by injection of I 125 followed by repeated injections in amounts equal to those excreted by the toads. Similar though less complete, determinations made on the African clawed toad, Xenopus laevis, and the Mexican axolotl, Ambystoma mexicanum.

Thyroidal uptake of radioactive iodine has been measured many times in several amphibian species (See Rosenkilde 1969 1974). A typical value for B. bufo is 18–20% of injected amount. Xenopus takes up much less (usually 2–4%). Axolotls in their natural occurring, larval (neotenous) form also have low iodine uptakes (1 5%). (Artificially) metamorphosed axolotls have varying levels of thyroid function from about nil with corresponding absence of thyroidal iodine uptake, up to values similar to those of other naturally metamorphosed urodeles (20–30%).

Total iodine concentration in plasma shows large interspecific variations. B. bufo usually have around 0.01 µg/ml (range 0.00–0.03), comparable concentrations are found in larval and metamorphosed axolotls. larval. 0.03, metamorphosed. 0.06 µg/ml, while Xenopus plasma contains 0.20 µg/ml. The difference only partly corresponds to the different iodine content of the food. Xenopus and larval axolotls are fed pieces of mussel (0.3 µg I/mg) while metamorphosed axolotls and B. bufo eat mealworms (usually less than µg I/100 mg).

Relative distribution of iodinated products was followed in isotopic equilibration experiments. In the thyroid, changes were only observed during the first 3–5 weeks, after which time the thyroid contained 25% (range 13–33%) of the labelled iodine, thyroxine containing 6% of thyroidal label. In plasma, equilibration was slower: PBI was 5% (range ...) of plasma I-125 after 1 month, 11% (6–15%) after 2 months, 22% (10–33%) after 3 months, and 37% (14–62%) after 4 months. Free thyroxine increased during the first months and was thereafter variable (usually 0.5–1.5% of the plasma label).

Total thyroxine concentration thus may be up to 50% of total iodine, 0.01 µg/ml, i.e. ... Free thyroxine is about 0.1 ng/ml. So, while the total thyroxine is low compared to mammals, free thyroxine is about five times the normal human concentration. This may explain the short biological half-life for I 125 thyroxine found in six series of determinations ... h (range 11 0–16 8)

References

ROSENKILDE, P. Coll Int C N R S 1969 177 287
ROSENKILDE, P. Fortschr Zool 1974, 22 99

D 48

...cation of Neurones in the Cat *Medulla oblongata* That are Activated by Hind Limb Group I Muscle—and by Skin Afferents

By H. JOHANSSON and H. SILFVENIUS. *Department of Physiology University of Umeå, Sweden*

The medullary relay of the hind limb group I path to the cerebral cortex is located in nucleus Z of Brodal and Pompeiano (1957), as shown by Landgren and Silfvenius (1971).

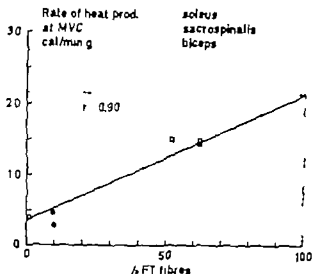


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Linear extrapolation suggests that a muscle composed exclusively of FT fibres wo... have a maximal heat production of 2.15 cal/min g, whereas a muscle containing slow tw... (ST) fibres only would have a maximum of 0 35 cal/min g.

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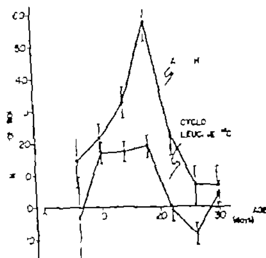
GOLDSPINK, G R E LARSON and R. E. DAVIES, Z vergl Physiol. 1970 66. 389–397

D 47

Metabolism of Iodine and Iodinated Thyroid Products in Amphibia

By P ROSENKILDE, Zoophysiological Laboratory A University of Copenhagen, 15 U... versitetsparken, Denmark

A survey of thyroid hormone dynamics in adult amphibia is attempted The most com... plete investigation was made on the common toad Bufo bufo I 131 uptake, total iodi...



1. Effects of GH on accumulation of 1B and ¹⁴C-cycloleucine of diaphragm normal rats of different ages. Hemidiaphragms were incubated for 90 min with and without bovine GH (5 µg/ml) in Krebs-Ringer bicarbonate buffer containing glucose (2.5 mg/ml), H-AIB (0.1 mM) and cycloleucine (0.1 mM), and the distribution ratios of the labelled amino acids were measured at the end of the incubation period described earlier (Hjalmarson and Ahrén, 1967a). Values presented are pooled means ± S.E. of 5 different experiments including diaphragms. The effect of GH is given as per cent of control

m young normal rats showed marked sensitivity to the stimulatory effect of GH. Studies have therefore been started to elucidate the sensitivity to GH of diaphragms from young normal rats of different ages. It can be seen from Fig. 1 that GH increases the uptake of the non-utilizable amino acids AIB (α-aminoisobutyric acid) and cycloleucine in diaphragms from 10, 14, and 18 day old rats; the effect being most pronounced in diaphragms from 18 day old rats. No significant stimulatory effect of GH was noticed in diaphragms from 6, ... and 30 day old rats. Thus, the present results demonstrate that the diaphragm muscle of normal rats is considerably sensitive to GH when excised from rats of certain ages. At present there is no clear explanation to the changes in sensitivity to GH in relation to age, and the present working hypothesis is that the changes in sensitivity might be due to altered pattern of endogenous secretion of GH paralleling the changes in feeding habits when the rats grow older

References

Hjalmarson, Å. and K. Ahrén, Acta endocr. (Kbh.) 1967 54 645–662.
Hjalmarson, Å. and K. Ahrén, Acta endocr. (Kbh.) 1967 b, 56, 347–358.

D 50

Three-component Output Analysis of Respiration during Rebreathing and Exercise in Man

By S ... Sten ... Pedersen, Michael Grunstein, Jakob Mertz, Joseph Milic-Emili and Poul Erik Paulev. Institute of Medical Physiology B, University of Copenhagen, Denmark and Department of Physiology, McGill University, Montreal, Canada

The CO₂ "sensitivity" of the respiratory control system has usually been referred to the increment in ventilation (V_E) per increment in P_{CO_2}. The results concerning CO₂ sensitivity

Recent findings further demonstrate that medullary axon-collaterals of the dorsal spino-cerebellar tract, DSCT monosynaptically activate group I relay cells in nucleus Z (Johans-son and Silfvenius 1973) Extended observations do however show that ipsilateral group I hind limb afferents also excite medullary neurones which presumably are not located in nucleus Z.

Recordings have been made from 13 group I and 3 skin activated cells, localized lateral to nucleus Z, and from 6 group I and 2 cutaneous cells found rostrolateral to the nucleus. The properties of the cells recorded from were similar to those of the nucleus Z. There were thus medullo-thalamic relay cells found among the neurones with the locations mentioned Evidence for DSCT axon-collateral activation of these group I relay cells was also found.

The results suggest that the cells localized lateral to nucleus Z are located in the rostral portion of the cuneate nucleus, and that the cells localized rostrolateral to nucleus Z belong to the hitherto functionally undefined nucleus X of Brodal and Pompeiano (1957).

DSCT would thus provide short latency information to a medullary nucleus classically considered to forward forelimb information (cuneate nucleus) and to a nucleus (X) anatomically known to project to the posterior cerebellum (Brodal and Torvik 1957). An anatomical confirmation of a hind limb input to the rostral portion of the cuneate nucleus would exclude the possibility that the unitary observation made is due to recordings from aberrant neurones.

References

BRODAL, A. and O POMPEIANO, *J Anat* 1957 *91* 438–454

BRODAL, A. and A TORVIK, *Arch Psychiat Nervenkr* 1957 *195* 550–567

JOHANSSON, H and H SILFVENIUS, *Act ph mol. scand.* 1973 Suppl. 396. 94

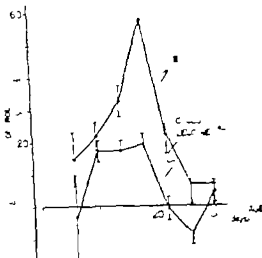
LANDGREN S. and H SILFVENIUS *J Physiol.* 1971 *18* 551 571

D 49

Sensitivity to Growth Hormone of Diaphragm Muscle from Young Normal Rats

By K. ALBERTSSON WIKLAND and O ISAKSSON *Department of Physiology University of Göteborg Sweden*

By few exceptions the metabolic effects and the mechanism of action of growth hormone (GH) have been studied in hypophysectomized animals. GH administrated *in vivo* or *in vitro* has a stimulatory effect on several metabolic events, like protein synthesis and the membrane transport of amino acids and sugars in different tissues from hypophysectomized animals. The early stimulatory effect of GH in *e g* diaphragms from hypophysectomized rats is after approximately 3 h followed by a "late inhibitory" effect of the hormone (Hjalmarson and Ahrén 1967 *b*). However other hormones than GH are also eliminated by hypophysectomy This opens the possibility that the effects of GH in hypophysectomized animals do not reflect the physiological function of the hormone Only small stimulatory effects or no effects at all have previously been observed in isolated tissues from normal rats. In preliminary experiments, however we observed that the diaphragms



Effects of GH on accumulation of and ^{14}C-cycloleucine of diaphragm mal rats of different ages. Hemidiaphragms were incubated for 90 min with and bovine GH (5 μg/ml) in Krebs-bicarbonate buffer containing glucose 5 μg/ml), ^{3}H-AIB (0.1 mM) and leucine (0.1 mM), and the distribution of the labelled amino acids were measured at the end of the incubation period described earlier (Hjalmarson and Ahrén,). Values presented are pooled means of 5 different experiments including diaphragms. The effect of GH is given cent of control.

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References

HJALMARSON, Å. and K. AHRÉN, Acta endocr. (Kbh.) 1967 a, 54, 645–662.
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D 50

Two-component Output Analysis of Respiration during Rebreathing and Exercise in Man

By SVEND STENVANG PEDERSEN, MICHAEL CRUNSTEIN, JAKOB MERTZ, JOSEPH MILIC EMILI and POUL-ERIK PAULEV. Institute of Medical Physiology B, University of Copenhagen, Denmark and Department of Physiology, McGill University, Montreal, Canada

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References

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D 49

Sensitivity to Growth Hormone of Diaphragm Muscle from Young Normal Rats

By K. Albertsson Wikland and O. Isaksson. *Department of Physiology, University of Göteborg, Sweden*

By few exceptions the metabolic effects and the mechanism of action of growth hormone (GH) have been studied in hypophysectomized animals. GH administrated *in vivo* or *in vitro* has a stimulatory effect on several metabolic events, like protein synthesis and the membrane transport of amino acids and sugars in different tissues from hypophysectomized animals. The early stimulatory effect of GH in e.g. diaphragms from hypophysectomized rats is after approximately 3 h followed by a "late inhibitory" effect of the hormone (Hjalmarson and Ahrén 1967 *b*). However other hormones than GH are also eliminated by hypophysectomy. This opens the possibility that the effects of GH in hypophysectomized animals do not reflect the physiological function of the hormone. Only small stimulatory effects or no effects at all have previously been observed in isolated tissues from normal rats. In preliminary experiments, however, we observed that the diaphragm

...entilation is the product of frequency and volume, and opposing changes in the two ...s may explain the above discrepancy.

...al volume (V_T or V_{TE}), frequency (f) and ventilation ($\dot{V}_E$) was measured in 6 subjects ...athing from a bag initially filled with 6–8 l of 7% CO_2 in 50% O_2 at different steady levels of cycling (600, 1200 and 1800 kpm/min). The data was recorded and com... on-line breath-by-breath with a system described earlier (Paulev 1971).

...e volume control. In all the subjects the increment in V_{TE} per increment in P_{ETCO_2} ($V_{TE}/\Delta P_{ETCO_2}$ or slope) during rebreathing was largest at rest and gradually falling at ...r work rates (fig. 1). *The time control.* The increment in frequency (Δf) per increment ...$_{CO_2}$ ($\Delta f/\Delta P_{ETCO_2}$) during rebreathing was smallest at rest in all subjects, rising more ...more following higher work rate. *Ventilation.* The ventilatory output varied un...ctably in the subjects, some showing an unchanged sensitivity ($\Delta \dot{V}_E/\Delta P_{ETCO_2}$) to CO_2 ...g rebreathing at higher levels. Other subjects showed a decreasing $\Delta \dot{V}_E/\Delta P_{ETCO_2}$ ... rest to the highest work rates. The peak value of the first time derivative of flow (A_1), the non-volume related respiratory output was measured, and shows at curvili... relation with increasing slope at increasing work rates (Fig. 1). This parameter is ...ured within the first 100 ms of inspiration, and thus A_1 is only slightly dependent upon ...me.

...he rate of change in P_{ETCO_2} remained constant in spite of the ever increasing V_E, which ...blishes a stable relationship between P_{ETCO_2} and medullary chemoreceptor P_{CO_2}, thus ...sing that P_{ETCO_2} may be as valid an input index for the CO_2-stimulus as the arterial ...r.

...he above mentioned conflicting reports concerning CO_2 sensitivity expressed as ($\Delta \dot{V}_E/$ ...$_{CO_2}$) during exercise may thus be an unnecessarily complicated concept, due to opposing ...nges in the 2 factors (f V_{TE}). Clarification of the problem requires separate measure...nts of the pacemaker and volume regulating system.

References

...ASEN, E. and M. NIELSEN, *Acta physiol. scand.* 1957. 39. 27–35.
...ON, A. B. Jr and B. A. BABCOCK, *J. appl. physiol.* 1962. 17. 874–876.
...RAM, J. B., W. W. PRYOR, E. B. PAGE and R. J. ATWELL, *J. clin. Invest.* 1951. 30. 503–516.
...XWORTHY, J. F. J., *U.S. Air Force Tech. Rept.* 1951. 6528. 479–485.
...LEV, P. E., *J. appl. Physiol.* 1971. 30. 165–172.

D 51

...netics of the Glomerular Ultrafiltration

By Ö. KÄLLSKOG, L.-O. LINDBOM, H. R. ULFENDAHL and M. WOLGAST. *Department of Physiology and Medical Biophysics, Biomedical Center, University of Uppsala, Sweden*

...A lot of problems are still to be solved about the kinetics of the glomerular filtration ...rocess. According to the results of Brenner and coworkers the general opinion seems to ..., that the filtration process is in equilibrium in the glomerulum. This should mean, that ...he net driving force at the end of the glomerular capillary is zero. The results of the present investigation show however a condition far from equilibrium.

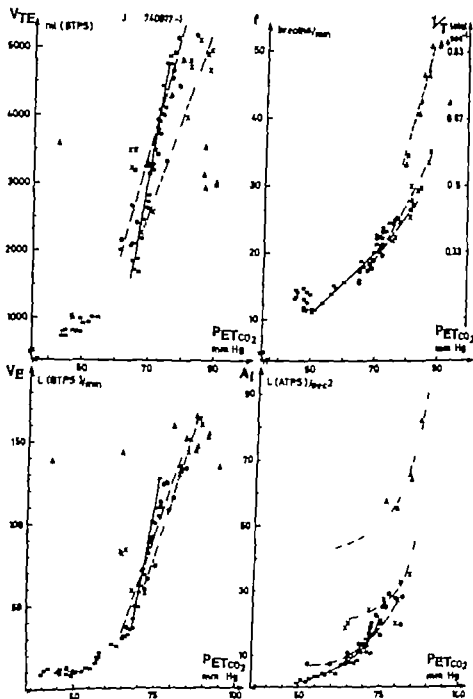


Fig. 1 The CO_2 response curves for expired tidal olume (V_T o V_{TE}), respiratory frequency (f), entilation (V_E) and inspiratory peak acceleration (A_I). The symbol denotes rebreathing during rest and the solid lines are tentative slopes during this condition Rebreathing during steady st te cycling is symbolized with O × Δ for 600, 1200, and 1800 kpm/min, respectively The related slopes and curves are: Dashed, short and long dashes, and small dashes.

during exercise have been diverging, some finding increased sensitivity (Hickam, Pryor Page and Atwell 1951), some finding decreased sensitivity (Muxworthy 1951), while others found it to be unchanged (Asmussen and Nielsen 1957 Craig and Babcock 1962).

The experiments were performed on adult Sprague Dawley rats and with micropunc techniques the single nephron glomerular filtration rate, the filtration fraction and oncotic and hydrostatic pressures in superficial nephrons and vascular structures v measured and calculated. The mean net driving pressure was about 20 mm Hg and the driving pressure at the end of the glomerular capillary was about 13 mm Hg. From experimental values it could mathematically be shown, that the glomerular filtration must not by necessity be correlated to the glomerular plasma flow as postulated by B ner Rather it seems to be a combined influence of hydrostatic and oncotic pressures w the glomerulum and the glomerular plasma perfusion rate. According to our results pre- and post-glomerular resistances regulate these parameters.

Reference

BRENNER, B M W M DEEN and C. R. ROBERTSON, *The physiological basis f glomerular ultrafiltn In kidney and urinary tract physiology* (ed. U Thurau). London 1974 P 336.

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From the Department of Experimental Medicine, Pharmacia AB, Uppsala, Sweden

Protein Concentration in Interstitial and Lymphatic Fluids from the Subcutaneous Tissue

By

Gianfranco Rutili and Karl E. Arfors

Received 24 February 1976

Abstract

Rutili, G. and K. E. Arfors. *Protein concentration in interstitial and lymphatic fluids from the subcutaneous tissue.* Acta physiol. scand. 1977 99 1–8.

The protein content of interstitial fluid and lymph from the same tissue was determined, fluid samples of nanoliter volumes being taken from the subcutaneous tissue of rabbits by micropuncture. I the same area lymph as collected from lymphatic vessels of 30–100 µm. An electrophoretic technique in polyacrylamide gels in glass capillaries as used for the analysis of proteins. The gels were stained and then scanned on microscope equipped with moving table and photomultiplier. The area under each protein band was calculated from the recorded densitograms. The method required only few nanolitres of fluid for analysis. Using solutions of know protein concentrations the relationship between the amount of protein and the area under the densitogram band as investigated. This relationship was found to be linear making it possible to quantify the protein content of both interstitial fluid and lymph samples. The interstitial fluid/plasma concentration ratios for albumin, transferrin, globulins and total proteins ere 0.42, 0.42, 0.32 and 0.37. The albumin globulin ratio in interstitial fluid as found to be 1.40 of the plasma value although the alues obtained for lymph were similar to those found for interstitial fluid. The similarity between the protein concentration of interstitial fluid and lymph indicated that the endothelial lining of the terminal lymphatics did not restrict the passage of macromolecules into the lymphatics.

Key words: Interstitial fluid, lymph, proteins, subcutaneous tissue, permeability

According to the classical Starling-Landis concept, capillary fluid balance is maintained by dynamic interaction between hydrostatic and osmotic forces acting across the capillary wall. These forces are considered to be of almost equal magnitude, therefore cancelling each other out. Efforts have been made during recent years to measure the numerical alues of the forces, but Wiederhielm (1971) has *pointed out that the literature on capillary fluid* balance still contains number of pparently conflicting results.

One of these conflicts results from the fact that while direct measurements of hydrostatic pressure (Richardson and Zweifach 1970) ha e been made possible by sensitiv micropressure devices (Intaglietta *et al.* 1970), the bulk of our information about interstitial fluid osmotic pressure is derived from measurement of lymph protein concentration. Experimental evidence that tissue fluid and lymph are identical in their protein composition is still lacking. Moreover in recent reports (Casley Smith and Bolton 1973 Casley-Smith

1976) it has been suggested that as a result of lymphatic compression, lymph is ultra-[f]
through the endothelium, thus increasing the protein concentration of the lymph [co]
pared to the interstitial fluid concentration.

Direct measurements of interstitial fluid protein concentration are difficult sinc[e]
minute amounts of fluid are available by sampling from connective tissue, and ade
analytical methods for protein determination in such small volumes have not been ava
until now. Indirect estimates of interstitial fluid protein concentration have been ma
determination of the partition of protein between plasma and the tissue space (Bers
al 1955, Walker *et al* 1960, Studer and Potchen 1968). These measurements, how
yield results which are intrinsically an expression of the average body or organ protein
centration, thus making comparison with lymph protein concentration difficult.

In the present investigation a technique making it possible to analyse the protein co[n]
of samples in the nanolitre range has been used to determine the protein concentratic
both interstitial fluid and lymph from the same tissue. The method consists of microp
ture for the collection of lymph and tissue fluid and an electrophoretic technique for
quantitative analysis of proteins in nanolitre samples of fluid.

Materials and methods

Prot[e]in determination

The prote[in] content of interst[i]tial fluid, lymph and plasma was determined by the disc-electrophor[esis] method [o]f Ornstein (1964) and Da[vi]s (1964). A micro-scale modification, and the practical details, h[ave] been extensively described by Smeds (1969). Certain further modifications were, however, made before [this] study. In brief, the electrophoresis was run in small glass capillaries of 3 μl volume (Drummond Sci. C[o.] Broomall, Penn., U.S.A.) with i.d. 200 μm containing a spacer and a runn[in]g polyacrylamide gel. The c[on]centration used was 5% in the spacer and 20% in the running gel. These concentrations [w]ere double th[ose] used by Smeds (1969) and resulted in increased resolution of the different proteins.

Ammoniumperoxydisulfate was used instead of riboflavin because it leads to more uniform cross-l[in]k[ing] in the polyacrylamide. The pulsed voltage over the gel was kept [a]t 175 V w[i]th a pulse rate of 500 H[z] a[nd] duty-cycle of 25%. After the run the gel was pushed out of the capillary and placed [i]n a solution of 20[%] sulfosalicylic acid for 15 min. Treatment of the gel with sulfosalicylic acid was performed to make the bin[din]g of Commassie Blue to the protein more stable. After staining for 15 min in a 0.2[%] solution of Co[m]massie Blue the gel was washed several times in a solution of acetic acid, methanol and distilled wat[er] (1:5:10) and stored for 1 h in the same solution.

A photomicrograph of the gel is shown in Fig. 1. A total of 15 protein bands were stained but only [al]bumin, transferrin and α_2 macroglobulin identified. The optical density of the [s]tained protei[n] d[is]cs [was] measured in [a] LEITZ ORTHOLUX microscope, f[i]tted with [a] photomultiplier (EMI KNOTT Typ[e] 6094 A, Knott Elektronik, Munich, West Germany). A logarithmic Integrator Recorde[r] (Vitatron, N.V. Dieren, Holland) gave recordi[n]gs of protein extinction. For measurement the gel was placed [i]n a slit (...5 0.4 mm) made in [a] brass plat[e] fixed o[n] [t]o a plexiglass plate. A cover glass was placed o[v]er the slit to kee[p] the gel wet during measurement. The gel was scanned [a]t 6 mm/min using powered microscope table. A[n] adjustable slit in the light path of the microscope permitted measurement of a specified width of the gel. A yellow filter (LEITZ 510) was also placed in the light beam. A densitogram is shown in Fig. 2.

To make quantitative measurements of the different proteins in the gel, the relationsh[i]p between the area under the curve and the amount of protein was investigated with solutions of standard h[u]m[an] serum proteins (Boeringwerke AG, West Germany) of known concentrations. The volumes of standard solutions used varied between 1.1 and 15.0 nl.

Sampling of interstitial fluid and lymph

The experiments were performed on 12 rabbits. The details of the micropuncture technique used for collection of interstitial fl[u]id have been described previously (Rutili [a]nd Arfors 1976).



Fig. 1. Photomicrograph of polyacrylamide gel electrophoresis of an interstitial fluid sample after staining with Coomassie Blue. The volume of the sample was 8.4 nl.

In summary, after careful dissection of the epidermal layer of the rabbit hindlegs, the subcutaneous tissue was exposed. In the dissected area the microcirculation was readily visible and lymphatics of 30 to 100 μm diameter could occasionally be observed. *Lymphatic vessels smaller than 30 μm and terminal sacs* could not be identified. Using a micromanipulator a small oil-filled glass pipette with a tip diameter of 8–10 μm was inserted into the tissue and fluid collected by slight aspiration produced by withdrawing 4–5 ml of air with a syringe connected to the pipette holder. The sample was placed in a teflon cup filled with paraffin oil so that the droplet assumed a spherical shape and its volume could be calculated by measurements of its diameter. The whole sample was then removed and placed onto the polyacrylamide gel. After electrophoresis the gel was analysed for its protein content. Since a total of 20 samples could be run in parallel the analysis was carried simultaneously with 4 lymph samples, 4 interstitial samples, 4 plasma and 8 standards for each rabbit. The volume of the standard proteins solution was determined in the same way as for the interstitial fluid samples.

Results

The relationships between the amounts of albumin, transferrin, globulins and total proteins, and the areas under absorption curves obtained from standard solutions were investigated.

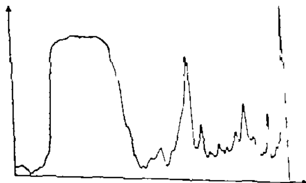


Fig. 2. Densitometric recording of the separation pattern of an interstitial fluid sample. The gel was scanned on a microscope equipped with a slowly moving table and photomultiplier for measurement of the optical density.

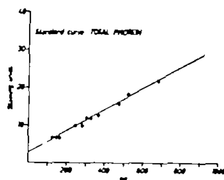
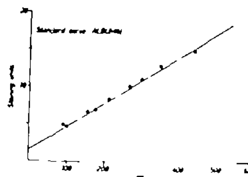
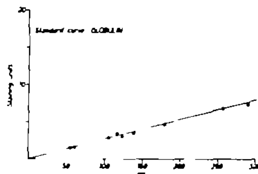


Fig. 3



F g 4



F g. 5

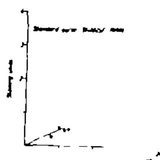


Fig. 6

F g. 3 4 5 6. Relationship between the mount of Total Proteins, Albumin, Total Globulins, Transferr n and the amount of Commassie Blue stain as measured from the optical density of the stained protei bands. The amount of stain is expressed as Staining Units. (Fo more details see text.)

The results are shown in Fig. 3 4 5 and 6. The area under each curve is expressed in staining units, which are calculated for each protein fraction using the formula S U I l/s E. I is the number of integration impulses, s is the reading given by a standard reference signal for the chart recorder which varies with the sensitivity adjustment of the recorder and E is the fixed value of the standard signal (0.2)

An almost linear relation for the different proteins was found within the range used in this investigation and the coefficient of variation of the analytical procedure was calculated and found to be less than 12 %. The protein concentrations of lymph (L) and interstitial fluid (I F) measured in the subcutaneous tissues of rabbits are given in Table I

T BLE I Plasma, lymph nd nterstitial fluid protei concentration (g/100 ml) of the subcutaneous t ssue of rabbits. The alues are mean ± S.D of I animals.

	T t. protei	Albumin	Globulin	Transferrin
Plasma	6 63	3 62	2.68	0..7
S D	0.58	0.36	0.40	0.03
Lymph	2.66	1.81	0.82	0 11
S.D	0.40	0.37	0.20	0 02
Int. fluid	2.45	1 60	0.86	0.11
S D	0.41	0.30	0.19	0.02

Interstitial fluid and lymph/plasma proteins. Concentration ratio of rabbit subcutaneous tissue.

	Tot protein	Albumin	Globulins	Transferrin	A/G
1	0 37	0.42	0.32	0 42	1 60
	0 06	0 08	0 07	0.09	0.22
	0 38	0 46	0 30	0.41	1 70
	0.06	0 08	0 09	0.08	0..0

significant difference between lymph and interstitial fluids was observed within the l error of the method.

able II the protein concentrations of I.F and lymph are compared with the respec lasma alues.

values for the albumin/globulin (A/G) of Table II are also expressed as ratios of the values. No difference was found between I.F and lymph.

similarity between interstitial fluid and lymph is shown more directly in Table III. es for albumin, globulins and transferrin determinations of the single experiment ven as a mean ratio between interstitial fluid and lymph concentration.

check the reliability of the micro-electrophoretic method for quantitative determina f proteins, plasma samples from 6 rabbits were analysed for their protein content by cro-zone electrophoretic standard method. The albumin/globulin ratios (A/G) ob- th the micro-zone electrophoretic method (I) were 1 40, 1 44 1.25 1.56, 0.81 0.98 those obtained with the micro-disk electrophoretic method (II) were 1 41 1.50, 1 15 0.92, 1 00 respectively The ratio I/II was 0.99 ±0.06.

Discussion

electrophoretic method used in this investigation for both qualitative and quantita- analysis has provided a means of measuring the protein concentrations of body fluid of nanolitre olumes. The relatively high coefficient of variation of the method should be considered in relation to the small volumes of the samples used for protein lysis (1 1 15 nl). Comparati analysis of the same samples by an independent method no significant difference between the methods and confirmed the validity of the sc electrophoretic technique. As the relationship between staining units and pro- concentration was linear quantitati determinations of protein concentrations in itial fluid, lymph and plasma were possible. The validity of the micropuncture sam- technique for the collection of tissue fluid has been discussed elsewhere (Rutili and Ar

III Albumin, globulins and transferrin interstitial fluid to lymph concentration ratio. The alues are mean S D of twelve rabbits.

in	Globulins	Transferrin
0 12	1 09 ± 0.21	1 02 ± 0 17

The alue of each single rabbit as obtained from 2 to 4 interstitial and lymph fluid samples.

fors, 1976) The overall findings of the study are consistent with the view that the sam| collected are of interstitial fluid. The time required for dissection of the epidermal layer; collection of fluid samples (≃5 min) is relatively short, and changes due to trauma and flammation are minimal.

The concentration of total protein in the interstitial fluid and lymph from the ral subcutaneous tissue was found to be lower than the concentration in plasma (1 3) values found are in agreement with those previously reported for the same tissue and spec Haljamäe and Freden (1970) found an interstitial fluid to plasma protein of 0.32 and Cc tice (1960) a lymph to plasma protein ratio of 0 41

Using nylon wicks to collect interstitial fluid, Aukland and Fadnes (1973) found an terstitial fluid to plasma protein ratio of 0 56 in rats which is somewhat higher than value found in this investigation. Our lower values may be due to a species difference. studies where interstitial fluid collected using wicks were directly compared with interst fluid collected by micropuncture (Rutili and Arfors, to be published), no significant ference was found between the two methods.

Of the 15–16 different proteins stained, only the albumin, transferrin and total globu concentrations were determined separately α_2-Macroglobulin could not be quantified curately because of its low concentration The concentrations of these protein fractions the interstitial fluid and in the lymph were identical (Table I) The similarity in composit between the two fluids suggests that the passage of macromolecules from the tissues to the lymphatics is not restricted by the endothelial lining of the terminal lymphatics. The morphological explanation of this "no-restriction concept was given by Leak and Burke (1968) and by Cliff and Nicoll (1970) In electron microscopic studies these workers have found "flaps and gaps" to be present in the endothelial layer of the terminal lymphatics permitting the passage of quite large molecules. Moreover they found anchoring filaments connecting the small lymphatics to the surrounding tissue, representing the mechanism responsible for opening the lymphatic capillaries in conditions of increased tissue pressure. The lack of a concentration gradient between the tissue fluid and the lymph fluid also indicates that the *net transport of macromolecules between the two compartments is, at steady-state, a convective rather than a diffusive process.* If this is a valid argument it will follow that the concentration of proteins in the tissue is, within their volume of distribution, evenly distributed. The capillary wall will thus represent the site of selectivity to molecular transport and the steady-state lymph/plasma ratio a measure of its permeability Garlick and Renkin (1970) arrived at a similar conclusion as a result of kinetic studies of the passage of different macromolecules from blood to lymph The hypothesis that lymph protein concentration is higher than interstitial fluid concentration (Casley Smith and Bolton 1973 Casley-Smith 1976), providing the driving force for osmotic flow from interstitial fluid into lymphatic capillaries, is not supported by the present results. The idea that net transport of proteins from interstitial space into lymph by an osmotic flow occurring as a result of a concentration gradient of the same proteins, has already been shown to be incompatible with the physical laws of osmotic flow (Michel 1974)

The albumin/globulin (A/G) ratio in tissue fluid and lymph was found to be higher than the A/G ratio of plasma by a factor of 1 6–1 7 The absolute values of the A/G ratio

interstitial fluid and lymph are not given in the results because of the large variation nd in the A/G ratio of plasma between different rabbits (Table III). The mean A/G ratio plasma was, however 1.35 compared to mean A/G ratio in tissue fluid of 2.20. A similar tissue fluid value (2.67) was found in the same tissue by Haljamäe and Freden (1970). higher A/G ratio of interstitial fluid relative to plasma indicates a higher permeability ened walls to albumin than to globulins. The transport of proteins across the microculation is, according to Lassen et al. (1974) a unidirectional process. The functional orphological structure in the capillary walls through which this process takes place has en described by Grotte (1956). From studies of the transcapillary passage of dextran of creasing molecular size, he suggested the presence in the capillary wall of pores of 35–45 radius, excluding molecules of the size of albumin, and fewer large pores of 120–350 Å radius through which the filtration of plasma could occur.

The effective filtration area of porous membrane available for a molecule is a function f the effective molecular radius (R_{ES}) and of the pore radius. Equations for the calculation the effective filtration area have been formulated by Renkin (1954). Using these equations nd assuming a R_{ES} for albumin of 35 Å and a R_{ES} for globulins of 50 Å, the A/G ratio ound in these experiments may be explained in terms of filtration through pores of 250–00 Å diameter in the capillary wall.

An alternative to the large pore hypothesis is vesicular transport. Endothelial vesicles ere firstly described by Palade (1953) after electron microscopic observation of the fine tructure of the capillary wall. Electron dense tracers such as Ferritin (R_{ES} 110 Å) or Gold R_{ES} 100 Å) injected into the circulation were found to be localized within the vesicles Karnovsky 1968) suggesting their participation in transporting material across the capillary all. Vesicular transport is subject to steric restriction (Garlick and Renkin 1970). Assuming a esicle radius of 250 Å (Bruns and Palade 1968, Casley-Smith 1968) globulins will be restricted by a factor 1.2 1.3 relative to albumin. This explains only in part the measured A/G ratio of 1.6–1.7 and the difference can only be explained by assuming an additional transport of albumin through the small pore system.

The identical C /C_p ratio of albumin and transferrin (molecular weight 70 000 and 88 000 respectively) is of interest considering the difference in the electrical charge of the two molecules (2.1 at pH 8.6, isoelectric points 4.9 and 5.9 respectively) and suggests that the permeability of vessels to proteins is a function of molecular size.

We are grateful to Mr. Ove Forsberg for the skilful technical assistance in the electrophoretic analyses of this study.

References

Aukland, K. and H. O. Fadnes, Protein concentration of interstitial fluid collected from rat skin by wick method. Acta physiol. scand. 1973. 88. 350–358.

Bunson, J. A., K. S. Kim and J. L. Bollman, Extravascular diffusion of proteins. Amer. J. Physiol. 1955. 182. 17 220.

Bruns, R. and G. Palade, Studies on blood capillaries. J. Cell. Biol. 1968. 37. 244–299.

Casley-Smith, J. R. The dimensions and numbers of small vesicles in blood and lymphatic endothelium and in mesothelium. J. Anat. 1968. 103. 202. 203. (Abstract.)

Casley-Smith, J. R., The functioning and interrelationship of blood capillaries and lymphatics. Experientia (Basel) 1976. 32. 1 L.

CARLEY SMITH, J. R. and T. BOLTON. The presence of large colloidal osmotic pressures across large pores. *Microvasc. Res.* 1973. *5*. 213–216.

CLIFF, W. J. and P. A. NICOLL. Structure and function of lymphatic vessels of the bat's wing. *Quart. J. exp. Physiol.* 1970. *55*. 112 1 1

COURTICE, F. C. Determination of protein fractions in small samples of plasma and of lymph in the rabbit. *Aust. J. exp. Biol. med. Sci.* 1960. *38*. 395–402.

DAVIS, B. J. Disc-electrophoresis. II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sc.* 1964. *121*. 404–426.

GARLICK, D. G. and E. M. RENKIN. Transport of large molecules from plasma to interstitial fluid and lymph in dogs. *Amer. J. Physiol.* 1970. *219*. 1595–1605

GROTTE, G. Passage of dextran molecules across the blood lymph barrier. *Act. ... kir. scand.* 1956. Suppl. 211. 1–84

HALJAMAE, H. and H. FREDEN. Comparative analysis of the protein content of local subcutaneous tissue fluid ... nd plasma. *Micro... sc. Res.* 1970. 2. 163–171

INTAGLIETTA, M., R. F. PAWULA and W. R. TOMKINS. Pressure measurements in the mammalian microvasculature. *Microvasc. Res.* 1970. *16*. ?94–300.

KARNOWSKY, M. J. The ultrastructural basis of capillary exchange. *J. gen. Physiol.* 1968. *52*. 64–95.

LASSEN, N. A., H. H. PARVING and N. ROSSING. Filtration as the main mechanism of overall transcapillary protein escape from the plasma. *Microvasc. Res.* 1974. *7*. I–iv. Editorial.

LEAK, L. V. and J. F. BURKE. Ultrastructural studies on the lymphatic anchoring filaments. *J. cell. Biol.* 1968. *36*. 1?9–149

MICHEL, C. C. The transport of solutes by osmotic flow with particular reference to the transport of proteins across the ... enous fenestrae of ... isceral capillaries. *Microvasc. Res.* 1974. *8*. 122 1 4.

ORNSTEIN, L. Disc-electrophoresis. I. Background and theories. *Ann. N.Y. Acad. Sc.* 1964. *121*. 321–349

PALADE, G. F. Fine structure of blood capillaries. *J. appl. Physiol.* 1953. *24*. 14 4 (Abstract.)

RENKIN, E. M. Filtration, diffusion, and molecular sieving through porous cellulose membrane. *J. gen. Physiol.* 1954. *38*. 225–243.

RICHARDSON, D. R. and B. W. ZWEIFACH. Pressure relationship on the macro- and micro-circulation of the mesentery. *Microvasc. Res.* 1970. *2*. 474–488.

RUTILI, G. and K. E. ARFORS. Fluorescein labelled dextran measurement in interstitial fl..id ... n studies of macromolecular permeability. *Microvasc. Res.* 1976. In press.

SMITH, S. Analysis of thy...oid proteins by means of micro disc-electrophoresis. *J. Chromatograph.* 1969. *44*. 148–160.

STUDER, R. and J. POTCHEN. The radioisotopic assessment of regional micro...ascular permeability to macromolecules. *Microvasc. Res.* 1971. *3*. 35–48

WALKER, W. G., R. S. ROSS and J. D. S. HAMMOND. Study of the relationship between plasma ...olume and transcapillary protein exchange usi...g [131]I-labelled album...n ...nd [131]I-labelled globulins. *Circulat. Res.* 1960. *8*. 1028–1040.

WIEDERHIELM, C. A. *Dynamics of capillary fluid ...xchange*. Proc. Int. Union of Physiol. Sciences. XXV Int. Congress, M...unich 1971. Vol. III. p. ..61–26...

Acta physiol. scand. 1977 99 9–18

From the Department of Psychiatry St. Goran Hospital and the Department of Histology Karolinska Institutet, Stockholm

Pineal Serotonin Metabolism in Non-Innervated Perinatal Glands before and after Intraocular Maturation Supersensitivity of Adrenoceptors that have never been Innervated

By

MARIA BÄCKSTRÖM, LARS OLSON and ÅKE SEIGER

Received 23 March 1976

Abstract

BÄCKSTRÖM, M., L. OLSON and Å. SEIGER. *Pineal serotonin metabolism in non-innervated perinatal glands before and after intraocular maturation. Supersensitivity of adrenoceptors that have never been innervated.* Acta physiol. scand. 1977 99 9–18.

Transplantations were made of fetal pineal glands (crown-rump length, CRL, 19–30 mm) or pineal glands from adult male rats to the anterior chamber of the eye of the rat. Studies were performed with regard to the importance of the age of the donor animal (and thereby the degree of maturation and innervation of the gland to be transplanted) for the possible development of denervation supersensitivity. The transplants were cultured in medium containing ^{14}C-serotonin. Increased production of ^{14}C N-acetylserotonin (NAcS) was used as the main criterion for β-adrenergic stimulation. 4 experimental groups were obtained by transplanting fetal or adult pineals to intact or sympathetically denervated eyes. In all 4 groups β_1 stimulation (KWD 2033 10^{-6} M) increased ^{14}C NAcS formation. The response to β-stimulation was significantly higher in denervated fetal pineal transplants than in innervated fetal transplants and thus demonstrating β-receptor supersensitivity. It was concluded that) the ability to respond to β-adrenoceptor stimulation ith increased ^{14}C-NAcS formation develops between the 18th and 20th day of gestation, b) transplants derived from fetal as ell as from adult rats can respond to β-adrenergic stimulation, c) this sensitivity also develops in oculo in transplants that at the time of transplantation lacked the capacity to increase their ^{14}C-NAcS formation in response to treatment ith β-agonist, d) denervation supersensitivity occurs in fetal transplants that become mature in sympathetically denervated eyes.

Key words: Pineal, transplants, serotonin, β-adrenergic, denervation supersensitivity, reinnervation.

The rat pineal gland metabolizes serotonin to N-acetylserotonin (NAcS) and melatonin (MEL) or 5-hydroxyindoleacetic acid (5-HIAA) (Wurtman *et al.* 1968). The activity of N-acetyltransferase (NAT), N-acetylating serotonin to NAcS (Weissbach *et al.* 1960), is controlled by β-adrenergic stimulation of the pineal noradrenoceptor mediated through increased intracellular production of cyclic adenosine 3',5'-monophosphate (cAMP) (Klein, Berg and Weller 1970). In our previous study homologous intraocular transplants from newborn rats were assayed 2.5–3.0 months following transplantation and were found to have

matured and to have become innervated by sympathetic nerves. They were responsive to external lighting conditions with pronounced increases of NAT (20 1) and hydroxyindole-O-methyl transferase (HIOMT) activities during dark hours as compared to light activities (Bäckström Olson and Seiger 1976) Similar findings have recently been reported by Moore (1975) Sympathetic denervation of the host eye eliminated the dark induced rise in HIOMT activity and greatly attenuated NAT enzyme variation (2 1). Cholinergic iris nerves have been shown to readily reinnervate eye chamber transplants that receive cholinergic fibers in their normal locus (Tsukahara, Jacobowitz and Laties 1972) The constant presence of cholinergic nerves in all host irides did not modify the dependence on an intact adrenergic innervation.

The persistent low amplitude NAT variation in denervated eye chamber pineal transplants was statistically significant One explanation for this variation could be the development of supersensitivity to circulating catecholamines in the transplants subsequent to denervation (Cannon and Rosenblueth 1949 Trendelenburg 1966) Development of denervation supersensitivity to β-adrenoceptor stimulation in rat pineal glands has been described (Deguchi and Axelrod 1972, 1973 Backstrom and Wetterberg 1973 Strada and Weiss 1974).

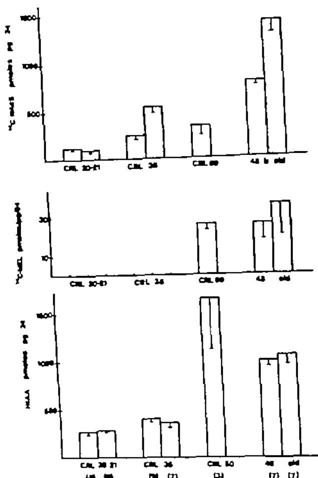
It was desired to investigate whether the degree of maturation and sympathetic innervation of the grafted pineals could influence the eventual status of indole metabolism. Would receptor supersensitivity in response to β-adrenoceptor stimulation develop in a pineal gland transplanted into a sympathetically denervated eye, at a stage prior to the sympathetic fiber development in the gland? Thus, would the receptor become supersensitive without ever having been contacted by its appropriate nerve supply?

Materials and methods

Animals. Albino rats (Sprague-Dawley) were fed food pellets and water *ad libitum* and kept one (pregnant rats) or five to a cage. Lights were on between 06 and 18 h. Pregnant rats, used for direct organ culture of fetal pineals, were decapitated between 10 and 16 h Uteri were quickly dissected out and kept on ice while preparations of tissues were performed. Pineal glands from fetal rats with CRL 20–21 mm, 50 mm and from 48 h old rats were each taken from one litter Pineal glands from fetal rats with CRL 35 mm were taken from two litters. The fetal or newly born rats were decapitated and using a dissection microscope the pineal glands were freed from adherent tissue. Pineal glands were stored in ice cold medium for 1 2 min before being introduced into the culture chamber Normal control rats were 300–350 g males, housed for 14 days on the same light dark cycle. Host animals were sacrificed by decapitation between 9.30 and 12.30 h The eyes were immediately removed and the transplants kept on ice during the dissection. Simultaneously processed *in situ* pineal glands were dissected out and stored in ice-cold incubation medium. All tissues were placed in the incubation chamber within approximately 3 min after decapitation.

Transplantations. Pineal glands from fetal rats with CRL 19–30 mm (18–20 days of gestation) and from adult male rats (150–200 g) were bilaterally transplanted into the anterior chamber of the eye, in adult recipient male rats. Pineals were rapidly dissected out from the donors, decapsulated and introduced into the anterior eye chamber through a slit in the cornea with a modified Pasteur pipette according to Olson and Malmfors (1970). All recipient animals (totally 33 rats) underwent unilateral sympathetic denervation by extirpation of either the left or the right superior cervical ganglion a few days before transplantation. The cholinergic innervation of the recipient iris was in all cases left intact. The taking and development of the transplants were followed *in situ* by repeated inspections through the cornea using a stereoscopic microscope under light ether anesthesia. The postoperative time ranged from 3 to 4 months.

Organ culture Pineal glands from fetal rats and newly born rats were preincubated for 1 2 h before the β_1-adrenoceptor agonist KWD 2033 (10^{-6} M) (Carlsson *et al.* 1975) was added as indicated in Fig. 1 Pineal transplants and *in situ* pineal glands from experimental and normal rats were preincubated for 2–4



[illegible] 1. Production of ^{14}C-serotonin derivatives by cultured immature rat pineal glands. ^{14}C-NAcS = ^{14}C [illegible]-Acetyl)serotonin. ^{14}C MEL = ^{14}C Melatonin, ^{14}C 5-HIAA = ^{14}C 5-Hydroxyindoleacetic acid. CRL val[illegible] [illegible] give crown-rump length in mm of the animals used. Empty and shaded bars indicate mean values o[illegible] [illegible]C-labelled metabolite formation in unstimulated controls of KWD 2033 (10^{-4} M) stimulated gland[illegible] [illegible]spectively, measured as picomoles of metabolite formed per pineal gland per 24 h culture period [illegible] [illegible]tandard error of the mean is indicated by vertical line at bar. The number of pineal glands cultured pe[illegible] [illegible]roup [illegible] presented [illegible] brackets below bars displaying ^{14}C 5-HIAA formation. [illegible] designate groups tha[illegible] [illegible]iffer significantly (p [illegible] 0.01) from their respective control groups.

[illegible] Following the preincubation period KWD 2033 (10^{-4} M) was added to some of the [illegible] as stated [illegible] Table 1 and Fig. 2. All pineals were cultured according to the method developed by Trowell (1959), mod[illegible] [illegible]ied by Rost (1965) and first elaborated for pineal organ culture by Klein and Weller (1970). The glan[illegible] were each cultured in 0.6 ml of BGJ$_b$-medium, Fitton-Jackson modification (Grand Island Biologic[illegible] Company). Bovine serum albumin (1 mg/ml), ascorbic acid (0.1 mg/ml) and 1-glutamine (0.3 mg/ml) [illegible] added on the day of the expt. The medium contained 0.25 mM ^{14}C 5-hydroxytryptamine (serotoni[illegible] creatinine sulphate (Radiochemical Center, Amersham), specific activity 54 mCi/mmol, diluted with [illegible] labelled serotonin (Fluka AG) to 4 mCi/mmol. No correction [illegible] feasible for the decrease in ^{14}C-serotoni[illegible] specific activity due to endogenous production of serotonin from 1-tryptophan contained in the cultu[illegible] medium. The culture chamber [illegible] continuously exposed to 95 [illegible] O_2 and 5 [illegible] CO_2 at 37°C. All tissues [illegible]

TABLE I Production of ¹⁴C serotonin derivatives by cultured pineal glands and transplants

Pineal types	Treatment		Metabolites in medium		
			N Acetyl 5-HT	Melatonin	5-HIAA
In situ normal	Control	(4)	152±32	338±67	3 930±155
	KWD 2033	(7)	828±146	656±61	2 556±473
In situ experimental	Control	(6)	167±30	294±45	3 120±148
	KWD 2033	(4)	664±215	688±138	2 904±528
All innervated transplants	Control	(16)	159±21	170±41	3 430±344
	KWD 2033	(13)	512±26	182±41	3 078±189
All denervated transplants	Control	(14)	186±27	87±22	3 406±409
	KWD 2033	(16)	1 1.8±96	332±79	2 945±377

Data are expressed as mean ± standard error of the mean of picomoles ¹⁴C labelled metabolite formed pineal per 24 h culture period, preceded by 1 h preincubation. N Acetyl-5-HT N-Acetylserotonin HIAA = 5-Hydroxyindoleacetic acid Numbers in brackets designate the number of pineal glands or tr plants cultured per group. KWD 2033 concentration was in all cases 10^{-4} M nd indicate gr that differ significantly in amounts of ¹⁴C metabolites formed as compared to their respective control gro $p < 0.05$ p 0.01 and $p < 0.001$ respectively

cultured for 24 h following addition of β-adrenoceptor agonist (Bäckstrom and Wetterberg 1973). M were collected and frozen at −20°C. The ¹⁴C-labelled serotonin metabolites were separated by thin-l chromatography as described by Klein and Notides (1969). Radioactivity was eluted in 95 ethano 0.1 M HCL Insta-Gel (Packard Instrument International S.A.) or Aquasol (New England Nucl were added and radioactivity determined by liquid scintillation (Packard Tri-Carb 3380, supplied th Absolute Activity Analyzer 544)

Doses of KWD 2033 were given as the final concentration of the base in culture medium

Statistical analyses were performed by pplication of Student's two-tailed t test for comparison of me

Results

In vitro observations All pineals survived transplantation into the anterior chamber of t eye regardless of the stage of development at operation. They became rapidly vasculariz from the iris of the host eye. The most immature pineals (CRL 19–21 mm) were appro mately 1 1 mm at operation and grew *in oculo* to a final size of approximately 2 1 5 m. Pineals from CRL stages 22–25 mm and 27–30 mm were initially somewhat larger a reached the same final size as the most immature pineals. Adult pineals were approximate 2 1.5 mm already at transplantation, and did not change size *in oculo* Denervat transplants were visibly smaller than innervated transplants regardless of donor age.

Immature pineals Pineal glands from the most immature fetal rats (CRL 20–21 mm formed ¹⁴C NAcS The production increased with maturity (Fig. 1). After addition KWD 2033 (10^{-4} M) the pineal glands from fetal rats with CRL 35 mm and 2 day old ra significantly increased their production of ¹⁴C NAcS (2.2 and 1 8 times respectively) compared to controls ($p < 0.01$ and $p < 0.001$ respectively)

There was no production of ¹⁴C MEL in pineal glands from fetal rats with CRL 20 21 mm or CRL 35 mm either untreated or following stimulation with KWD 2033 (10^{-4} M (Fig. 1). Pineal glands from fetal rats with CRL 50 mm and from 2 day old rats produce minimal amounts of ¹⁴C MEL and this production was not altered by addition of drug.

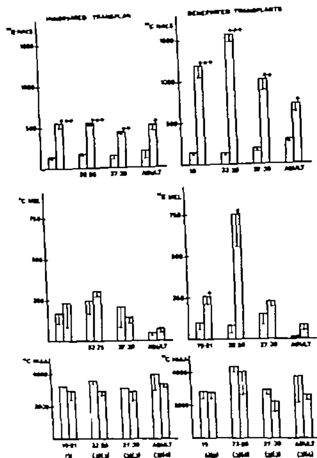


Fig. 2. Production of ^{14}C-serotonin derivatives by cultured innervated and denervated rat pineal transplants derived from donor rats of various ages. The left part of the figure shows metabolite formation in innervated transplants, the right shows denervated transplants. ^{14}C NAcS = ^{14}C-N-Acetylserotonin, ^{14}C MEL = ^{14}C Melatonin, ^{14}C-HIAA = ^{14}C 5-Hydroxyindoleacetic acid. Numbers below bars give crown-rump length (CRL) in mm of transplant donor animals. Empty and shaded bars illustrate mean values of ^{14}C-labelled metabolite formation in unstimulated controls or KWD 2033 (10^{-4} M) stimulated transplants respectively, measured as picomoles of metabolites formed per transplant per 24 h culture period. Standard error of the mean is indicated by vertical line in bar. The number of pineal transplants cultured per group is presented in brackets below the bars which display ^{14}C 5-HIAA formation. and designate groups that differ significantly from their respective control groups (p 0.05, p 0.01 and 0.001 respectively).

The production of ^{14}C 5-HIAA also rose with age, reaching 25–30% of adult levels in 48 h old pineals (Fig. 1). KWD 2033 (10^{-4} M) did not affect the formation of ^{14}C 5-HIAA.

Normal mature and transplanted pineals. *In situ* pineal glands from normal rats and experimental rats (carrying bilateral eye chamber transplants) produced amounts of ^{14}C NAcS, ^{14}C MEL and ^{14}C 5-HIAA that did not differ statistically from each other when controls or KWD 2033 (10^{-4} M) treated groups were compared. In both types of glands KWD 2033 increased the production of ^{14}C NAcS (5.4- and 4.0-fold respectively) and ^{14}C

MEL (1 9- and 2.3-fold respectively) ($p < 0.05$) but did not alter ^{14}C 5-HIAA formation (Table I)

When all non-stimulated transplants from the different donor groups were compiled there was no difference between those that were sympathetically innervated and those that were denervated, in the formation of ^{14}C labelled NAcS, MEL or 5-HIAA per transplant (Table I). β-stimulation by KWD 2033 (10^{-4} M) caused a 2.7 times larger increase in ^{14}C NAcS production in denervated transplants than in innervated transplants ($p < 0.001$) In the denervated group KWD 2033 increased ^{14}C NAcS formation 6 1 times (Table I). This large ^{14}C NAcS increase, induced by KWD 2033 was significantly higher than in the *in situ* glands of the experimental rats. KWD 2033 (10^{-4} M) also caused a 3 8-fold increase in the formation of ^{14}C MEL in denervated transplants ($p < 0.01$) In innervated transplants there was no increase in ^{14}C MEL formation following KWD 2033 addition (Table I).

When innervated and denervated transplants were analyzed with respect to the maturity of the donor animal it was found that the three different fetal groups were approximately equivalent (Fig. 2) while transplants obtained from adult donors were found to increase their ^{14}C NAcS formation less in response to β-stimulation. They also had lost much of their ^{14}C MEL synthesizing capacity ^{14}C 5-HIAA formation in transplants was not affected by denervation or drug treatment (Table I and Fig. 2).

Discussion

In the denervated eye chamber pineal transplants a low amplitude variation of NAT enzyme activity persisted (Bäckström, Olson and Seiger 1976) This might have been due to development of denervation supersensitivity of the transplants, which might have responded to circulating catecholamines. The eye chamber transplantation technique also offered a possibility to study such a phenomenon in very immature fetal pineal glands that had not been innervated by the time of operation and where ganglionectomy would have posed technical difficulties. It may thus be possible that these fetal tissues develop an adrenergic receptor supersensitivity without ever having been in contact with their appropriate nerves.

Culture medium content of the 3 ^{14}C-serotonin metabolites NAcS, MEL and 5-HIAA (5-hydroxyindoleacetic acid) were assayed to obtain an estimate of NAT HIOMT and monoamine oxidase (MAO) enzyme activities respectively Determination of ^{14}C 5-HIAA was considered of importance to ascertain the specific adrenoceptor effect ofthe agonist and exclude a mere inhibition of serotonin deamination. When this metabolic pathway which alternates with N-acetylation of serotonin, is inhibited a "mass-action" shunt over to N acetylation has been suggested (Axelrod, Shein and Wurtman 1969 Shein 1971). A newly synthesized β_1-adrenoceptor agonist KWD 2033 (Carlsson *et al* 1975) was chosen for the stimulation of pineal adrenoceptor mediated N-acetylation of ^{14}C-serotonin. KWD 2033 has recently been shown to have maximal efficacy and potency for stimulation of rat pineal gland β-adrenoceptors in organ culture (Bäckström 1976) Metabolite production was measured in the culture medium, calculated per pineal, and no compensation was made for the differences in amount of tissue between *in situ* pineal glands and transplants.

Ontogenetic development of serotonin metabolism. In earlier works pineals from fetal rats have been reported to lack responsive adenyl cyclase which constitutes part of the proposed pineal postjunctional adrenergic receptor (Weiss 1971). However pineal glands from fetal rats with CRL 35 mm (about 20–21st day of gestation) are found to double their ^{14}C NAcS formation in response to addition of the directly acting β-adrenoceptor agonist KWD 2033. This response was not observed in pineal glands from the most immature fetal rats with CRL 20–21 mm (about 17–18th day of gestation). NAT enzyme activity has been detected as early as 4 days before partus. The initially high values of NAT decreased markedly around 7 days of age (Ellison, Weller and Klein 1972). Similarly in comparison with mature pineal glands a higher unstimulated level of ^{14}C NAcS was formed around and 2 days subsequent to birth as seen in our present cultures. Our findings are compatible with the report of Weiss and Strada (1972) revealing a large increase of rat pineal phosphodiesterase activity between day 8 and 16 post partum. An increased breakdown of cAMP would lower the stimulation of postjunctional biochemical events required for maintaining NAT activity. HIOMT activity is hardly detectable during the first 10–12 days post partum in the rat pineal but then rises sharply to reach adult values at 34–50 days of age (Zweig and Snyder 1968, Snyder 1968, Klein and Lines 1969). A diurnal variation of HIOMT activity has first been detected in 39 day old rats (Klein and Lines 1969). Thus, the present absence or extremely low values of MEL production in very young rats were in good agreement with earlier reports. Low levels of MAO enzyme activity which is responsible for the deamination of serotonin to 5-HIAA, have been observed in pineals of newborn rats (Zweig and Snyder 1968, Snyder 1968). In the present organ cultures ^{14}C 5-HIAA formation was noted when pineals were explanted 4 days before birth, at a stage when pineals are not yet sympathetically innervated (Machado 1971). The ^{14}C 5-HIAA was thus formed extraneuronally probably in the pinealocytes.

Transplantations. Normal adult *in situ* pineal glands were cultured in the same experiment as pineal transplants and *in situ* pineal glands from experimental animals (carrying bilateral ocular transplants and submitted to unilateral superior cervical ganglionectomy). Pineal glands from normal adult rats did not differ in their production of ^{14}C-labelled NAcS, MEL or 5-HIAA as compared to the *in situ* pineal glands from experimental rats either unstimulated or β-stimulated with KWD 2033. This shows that the one remaining superior cervical ganglion supplied the *in situ* pineal glands with sufficient sympathetic nerves to ensure a normal adrenergic response. It also follows that the eye chamber transplants did not influence the activity of the *in situ* pineal gland to any significant extent.

Denervated transplants from fetal donors gave a higher increase of ^{14}C-NAcS production following β-stimulation with KWD 2033 than did innervated β-stimulated transplants taken from rats of corresponding ages indicating the development of supersensitivity to aminergic stimulation. This phenomenon was less pronounced in grafted pineals from adult donors although they were the only transplants which were actually denervated. Innervated grafts from all age groups responded to β-stimulation with an increased ^{14}C NAcS production of about the same magnitude as normal pineal glands. ^{14}C MEL formation in transplants was lower than in the *in situ* pineal glands both in denervated and innervated transplants. It was shown by Bäckström, Olson and Seiger (1976) that denervation of transplants caused

an extinction of HIOMT diurnal variation. Thus, eye chamber transplants behaved similarly to *in situ* pineal glands in that they are also dependent on an intact sympathetic innervation for their diurnal HIOMT variation (Axelrod, Wurtman and Snyder 1965). From the present experiments it can be seen that 24 h incubation with a directly acting β-adrenoceptor agonist could increase the production of ^{14}C MEL in denervated transplants but not in innervated transplants, although the latter displayed a dark induced rise in HIOMT activity *in oculo* The reason for this discrepancy is not readily explained. Nagle, Cardinali and Rosner (1973) found that intraperitoneal injections of noradrenaline caused significant increase in pineal HIOMT activity only in ganglionectomized rats and did not affect enzyme activity in intact animals. They interpreted these findings as a phenomenon of denervation supersensitivity The present observations on ^{14}C MEL formation in denervated transplants might be similarly explained Pineals from adult donors grafted to sympathetically innervated eyes loose some of their capacity to form ^{14}C MEL in spite of restoration of the sympathetic innervation. It is possible that the capacity of pineal grafts from adult donors to survive as functional eye chamber transplants is less than for fetal tissues.

Innervated and denervated transplants produced ^{14}C 5-HIAA in equally large amounts as the *in situ* pineal glands. Total MAO activity (measured in homogenates) has been reported to fall 30–50 %, following denervation of rat pineal glands (Snyder Fischer and Axelrod 1965 Håkansson and Owman 1966, Snyder 1968) mainly through elimination of MAO-A isoenzyme activity (Johnston 1968) which is predominantly contained in the sympathetic nerve terminals in the rat pineal (Goridis and Neff 1971 Neff *et al* 1974). Serotonin deamination was reported to decrease 70 % as compared to normal pineals when measured in homogenates of chronically denervated pineal glands (Goridis and Neff 1971). Any denervation induced decrease in ^{14}C 5-HIAA formation was not revealed in the present experiment This might be explained by a change of enzyme affinity for serotonin within the fraction of MAO enzyme remaining despite denervation. It can be noted that Håkansson and Owman (1965) found no decrease in pineal gland MAO activity for 21 days post denervation when ^{14}C-serotonin was used as substrate for incubations of intact glands. Bäckström and Wetterberg (1973) showed that chronically denervated *in situ* pineals cultured for 24 h in medium containing 0.25 mM ^{14}C serotonin, produced as much ^{14}C 5-HIAA as did normal *in situ* pineal glands in culture.

We conclude that pineal glands transplanted into sympathetically innervated host eyes become innervated regardless of the state of innervation or lack of such at the time of operation. The present experiments lend support to the suggestion that denervated eye chamber pineal transplants might become supersensitive to aminergic stimulation and that this supersensitivity could explain the discreet NAT enzyme alternation between dark and light that was seen in denervated transplants. It is not necessary for a normally sympathetically innervated tissue ever to have been innervated to develop a "denervation" supersensitivity to sympathomimetic amines.

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References

...o, J. H., M. Shein and R. J. W... tman, Stimulation of C14-melatonin synthesis from C14-tryptophan by noradrenaline in rat pineal in organ culture. *Proc. nat. Acad. Sci.* (Wash.) 1969, 62, 544–549.

...a, J., R. J. W... tman and S. H. Snyder, Control of hydroxyindole O-methyltransferase activity in rat pineal gland by environmental lighting. *J. biol. Chem.* 1965, 240, 949–954.

...sön, M., Effects of select... β-adrenoceptor ...gonists on rat pineal gland serotonin metabolism. ... To be published.

...sön, M., L. Olsen and Å. Seiger, N-acetyltransferase and hydroxyindole-O-methyltransferase ...ity in intraocular pineal transplants. Diurnal rhythm as evidence for ... functional sympathetic ...nergic innervation. *Acta physiol. scand.* 1976, 96, 64–71.

...n, M. and L. Wetterberg, Increased N-acetylserotonin and melatonin formation induced by ...ephrine in rat pineal gland organ culture ... β-adrenergic receptor mechanism. *Acta physiol. scand.* 1973, 87, 11–120.

...n, W. B. and A. Rosenblueth, *The supersensitivity of denervated structures.* Macmillan, New ...rk 1949.

...son, E. H. Pearson, G. Santl... lson, H. Schröter and K. Wetterlin, *Deutsche Offenlegungsschrift* ...3 ...501–751.

...m, T. and J. Axelrod, Induction and superinduction of serotonin N-acetyltransferase by adrenergic ...gs and denervation in rat pineal organ. *Proc. nat. Acad. Sci.* (Wash.) 1972, 69, 2208–2211.

...m, T. and J. Axelrod, Superinduction of serotonin N-acetyltransferase and supersensitivity of ...nyl cyclase to catecholamines in denervated pineal gland. *Mol. Pharmacol.* 1973, 9, 612–618.

...son, N. J., L. W... ller and D. C. Klein, Development of ... circadian rhythm in the activity of pineal ...tonin N-acetyltransferase. *J. Neurochem.* 197... 19, 1335–1341.

...son, C. and N. H. Ne..., E...idence for a ...pecific monoamine oxidase associated ...ith sympathetic ...rves. *Neuropharmacol.* 1971, 10, 557–564.

...sen, B. and C. ... Owman, Effect of denervation and enzyme inhibition on DOPA decarboxylase ...d monoamine oxidase act...ities of rat pineal gland. *J. Neurochem.* 1965, 12, 417–429.

...sen, B. and C. ... Owman, Pineal dopa decarboxylase and monoamine oxidase activities as related ...o the monoamine stores. *J. Neurochem.* 1966, 13, 597–605.

...n, J. P., Some observations upon ...new inhibitor of monoamine oxidase in brain ...ue. *Biochem. Pharmacol.* 1968, 17, 1285–1297.

...n, D. C., G. R. Berg and J. W... ller, Melatonin synthesis: Adenosine 3',5'-monophosphate and norepinephrine stimulate N-acetyltransferase. *Science* 1970, 168, 979–980.

...n, D. C. and S. V. Lines, Pineal hydroxyindole-O-methyltransferase activity in the growing rat. *Endocrinol.* 1969, 84, 1523–1525.

...n, D. C. and A. No...tons, Thin-layer chromatographic separation of pineal gland derivatives of serotonin-14C. *Anal. ...Biochem.* 1969, 31, 480–483.

...n, D. C. and J. W... ller, Input and output signals in ... model neural ...ystem: The regulation of melatonin production in the pineal gland. *In Vitro* 1970, 6, 197–204.

...mann, A. B. M., Electron microscopy of developing sympathetic fibres in the rat pineal body. The formation of granular vesicles. In: *Progr. in Brain Res.* (Ed. O. Eränkö). 1971, 34, 171–185.

... R. Y., Pineal transplant ...to the anterior chamber of the eye: E...idence for functional reinnervation. *E...p. Neurol.* 1975, 49, 617–6...1.

...n, C. A., D. P. Cardinali and J. M. Rosner, Retinal and pineal hydroxyindole-O-methyltransferases in the rat: Changes following cervical sympathectomy, pinealectomy or blinding. *Endocrinol.* 1973, ...1550–1564.

..., N. H., R. Y. T. Y...n, C. Goridis and D. Baller, The metabolism of indolealkylamines by type A and B monoamine oxidase of brain. I. ...*Advances in Biochem. Ps...hopharmacol.* Raven Press, New York 19... 11, 51–58.

...n, L. and T. Malmfors, Growth characteristics of adrenergic nerves in the adult rat. Fluorescence ...istochemical and 3H-noradrenaline uptak... studies using tissue transplantations to the anterior chamber of the eye. *Acta physiol. scand.* 1970, Suppl. 348, 1–112.

...r, L. G., Bone resorption in tissue culture. Factors influencing the response to parathyroid hormone. *J. ...in. Invest.* 1965, 44, 103–116.

...ers, H. M., Control of melatonin ...ynthesis by noradrenaline in rat pineal organ cultures. In: Wolsten-

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References

a, J. H. M. Smith and R. J. W... tman, Stimulation of C[14]-melatonin synthesis from C[14]-trypto- by noradrenaline in rat pineal in organ culture. *Proc. nat. Acad. Sci. (Wash.)* 1969. *62* 544–549
o, J. R. J. W... ma and S. H. S. ... Control of hydroxyindole 0-methyltransferase act... ity : rat pineal gland by environmental lighting. *J. biol. Chem.* 1965. *240*. 949–954.
son, M. *Effects of selective β-adrenoceptor agonists on rat pineal gland serotonin metabolism* T be published.
son, M. L. Olson and A. Seiger. N-acetyltransferase and hydroxyindole-0-methyltransferase ty in intraocular pineal transplants. Diurnal rhythm as evidence for functional sympathetic ergic innerv... tion. *Acta physiol. scand.* 1976. *96*. 64–71
son, M. and L. W... tte... Increased N-acetylserotonin and melatonin formation induced by phetamine in rat pineal gland organ culture ... β-adrenergic receptor mechanism. *Acta physiol.* / 197... *87* 113 120
... W. B. and A. Rosenblueth. *The supersensitivity of denervated structures.* Macmillan, New . 1949
... E. H. Person. G. S. ... H. Schröter and K. Wetterlin, *Deutsche Offenlegungsschrift* ..503 751
... T. and J. Axelrod, Induction and superinduction of serotonin N-acetyltransferase by adrenergic ... and denervation in rat pineal organ. *Proc. nat. Acad. Sci. (Wash.)* 1972. *69*. 2208–2211
... T. and J. Axelrod, Superinduction of serotonin N-acetyltransferase and supersensitivity of ...l cyclase to catecholamines in denervated pineal gland. *Mol. Pharmacol.* 1973. *9*. 612–618.
... N. J. L. W... and D. C. Klein, Development of circadian rhythm in the activity of pineal ...nin N-acetyltransferase. *J. Neurochem.* 1972. *19*. 1335–1341
... C. and N. H. Neff. Evidence for specific monoamine oxidase associated with sympathetic ... *Neuropharmacol.* 1971. *10*. 557 564
... R. and C. ... O...man, *Effect of denervation and enzyme inhibition on DOPA decarboxylase ... monoamine oxidase activities of rat pineal gland.* *J. Neurochem.* 1965. *12*. 417–429
... R. and C. Owman, Pineal dopa decarboxylase and monoamine oxidase activities as related ...he monoamine stores. *J. Neurochem.* 1966. *13*. 597–605.
... J. P. Some observations upon ... new inhibitor of monoamine oxidase in brain tissue. *Biochem. ...armacol.* 1968. *1*. 1285–1297
D. C. G. R. Berg and J. W... Melatonin ...thesis. Adenosine 3 5 monophosphate and nor...nephrine stimulate N-acetyltransferase. *Science* 1970. *168*. 979–980.
... D. C. and S. V. Lines. Pineal hydroxyindole-0-methyltransferase activity in the growing rat. *Endo-* ... 1969. *84*. 15... 15..3
... D. C. and A. Notides. Thin-layer chromatographic separation of pineal gland derivatives of sero-...nin-[14]C. *Anal. Biochem.* 1969. *31*. 480–483
... D. C. ... J. W... Input and output signals in ... model neural system. The regulation of mela-...nin production in the pineal gland. *In Vitro* 1970. *6*. 197 204
... A. B. M. Electron microscopy of developing sympathetic fibres in the rat pineal body. The ...rmation of granular ...sicles. In: *Progr. in Brain Res.* (Ed. O. Eränkö) 1971. *34*. 171 185
R. ... Pineal transplant... to the anterior chamber of the eye: Evidence for functional reinnervation. ...ocr. 1975. *49*. 417 4..1
C. A. D. P. C. ...sal and J. M. Rosner, Retinal and pineal hydroxyindole-0-methyltransferases ... the ...t. Changes following cervical sympathectomy, pinealectomy or blinding. *Endocrinol.* 1973. ... 50 1564
... N. H. H. Y. T. Y. ... C. Goridis and D. Bralek. The metabolism of indolealkylamines by type A ... B monoamine oxidase of brain. ... *Advances in Biochem. Psychopharmacol.* Raven Press, New York. ... 38
... L. and T. Malmfors, Growth characteristics of adrenergic nerves in the adult rat. Fluorescence ...ochemical and [3]H-noradrenaline uptake studies using tissue transplantations to the anterior chamber ... the eye. *Acta physiol. scand.* 1970. Suppl. 348. 1 112
... L. G. Bone resorption in tissue culture. Factors influencing the response to parathyroid hormone. *J. Cell. Invest.* 1965. *44*. 103 116.
... H. M. Control of melatonin synthesis by noradrenaline in rat pineal organ cultures. In: Wolsten-

holme, G. E. W. and J. Knight (eds) *The Pineal gland.* Ciba Foundat. Sympos. Churchill Livingstone, Edinburgh and London. 1971 197 207

SNYDER, S. H., J. FISCHER and J. AXELROD, Evidence for the presence of monoamine oxidase in sympathetic nerve endings. *Biochem. Pharmacol.* 1965 *14* 363–365

SNYDER, S. H. Development of enzyme activities and a circadian rhythm in pineal gland serotonin. Evidence for a nonretinal pathway of light to the pineal gland of newborn rats. *Advanc. Pharmacol.* 19[illegible] Part A. *6.* 301–305

STRADA, S. J. and B. WEISS, Increased response to catecholamines of the cyclic AMP system of rat pineal gland induced by decreased sympathetic activity. *Arch. Biochem.* 1974 *160* 197 204

TRENDELENBURG, U. I. Mechanisms of supersensitivity and subsensitivity to sympathomimetic amines. *Pharmacol. Rev.* 1966. *18* 629–640

TROWELL, O. A., The culture of mature organs in a synthetic medium. *Exp. Cell Res.* 1959 *16* 118–14[illegible]

TSUKAHARA, S., D. JACOBOWITZ and A. M. LATIES, Cholinergic reinnervation of iris transplants to the anterior chamber of the eye. *Amer. J. Ophthalmol.* 1972. *73* 394–399

WEISS, B., Ontogenetic development of adenyl cyclase and phosphodiesterase in rat brain. *J. Neurochem.* 1971 *18* 469–477

WEISS, B. and S. J. STRADA, Neuroendocrine control of the cyclic AMP system of brain and pineal gland. *Advanc. Cyclic Nucleotide Res.* 1972. *1* 357 374

WEISSBACH, H., B. G. REDFIELD and J. AXELROD, Biosynthesis of melatonin. Enzymatic conversion of serotonin to N-acetylserotonin. *Biochim. biophys. Acta* (Amst.) 1960. *43* 352 353

WURTMAN, R. J., F. LARIN, J. AXELROD, H. M. SHEIN and K. ROSASCO, Formation of melatonin and 5-hydroxyindole acetic acid from ¹⁴C tryptophan by rat pineal glands in organ culture. *Nature* (Lond.) 1968. *217* 953–954

ZWEIG, M. H. and S. H. SNYDER, *The development of serotonin and serotonin-related enzymes in the* pineal gland of the rat. *Communic. Behav. Biol.* 1968. Part A. *1* 103–108

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Local Reflex in Microcirculation in Human Skeletal Muscle

By

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Abstract

HENRIKSEN, O. and P. SEJRSEN. *Local reflex in microcirculation in human skeletal muscle.* Acta physiol. scand. 1977. 99. 19–26.

The effect of venous stasis of 40 mmHg upon blood flow in human skeletal muscle was studied in four normal subjects and in two chronically sympathectomized patients. Blood flow in skeletal muscle was measured by the local ^{133}Xenon washout technique. Blood flow decreased about 30 per cent during venous stasis of 40 mmHg. In "passive vascular bed" induced by means of histamine, blood flow decreased only by 16 per cent, indicating that the decrease in blood flow is due to a vasoconstrictor response to increase in vascular transmural pressure. The vasoconstrictor response was unaffected by spinal sympathetic blockade, but was blocked in areas infiltrated with lidocaine or with phentolamine. The vasoconstrictor response was present in the nonoperated limbs used as control, but abolished in the denervated areas in the two chronically sympathectomized patients. The findings strongly suggest that the vasoconstrictor response in skeletal muscle is due to a local nervous mechanism involving adrenergic fibres. Thus a local reflex mechanism, most likely a sympathetic axon reflex, seems to be present in human skeletal muscle as in cutaneous and subcutaneous tissue. This indicates that about 45 per cent of the change in total vascular conductance, when a person changes from supine to upright position, is due to this local reflex mechanism operating independently of the central nervous system.

Blood flow in human skeletal muscle decreased about 50 per cent when the leg was placed in dependent position (Bälldin *et al.* 1971, Amery *et al.* 1973). This vasoconstrictor response to increase in vascular transmural pressure has also been demonstrated in human subcutaneous adipose tissue (Henriksen, Levin Nielsen and Paaske 1973) and human cutaneous tissue (Henriksen *et al.* 1973). The vasoconstrictor response has been shown to be due to a local sympathetic reflex mechanism in these tissues (Henriksen and Abner 1975, Henriksen 1976 a, b, c, and d, Henriksen and Sejrsen 1976 a).

The purpose of the present study was to investigate whether there is a local reflex mechanism in the microcirculation in human skeletal muscle also. If so, the local reflex mechanism might account for a great deal of the total change in vascular conductance during changes from supine to upright position.

Methods

The experiments were ca ried out n four healthy subjects and two chronically ympathectomized paties operated because of ma ual hyperhidrosis.

Room temperat re was abo t 22 C and remained constant d ri g the in estigations. Blood flow a th a terior tibial muscle was estimated by the local ^{133}Xenon washout techn que (Lassen, Lindbjerg and Munk 1964). The γ-emission was detected by means of NaI(Tl) sci tillation detectors, collimated to record o from the d stal half of the labelled area in order to id nterference f om act ity em tted by Xenon ccumulating in fat tissue alo g the eins. The measurements were started 20 min p i n order t minimiz the nterference of th njection tra ma. Increase in ascula transm ral pressure was nduced by ven stasis. A cuff was placed on the th gh r the upper rm, d the ^{133}Xenon washout at constant, k was measured co secut ely 1) w th the cuff deflated, $k_{(pre)}$ 2) with the cuff inflated t 40 mmHg, k and 3) with the cuff deflated, k_{ref} Each period of measurement lasted bout 3 m n, d the count ra was printed out every 10 o 20 s.

I *Normals*

( ) *C t al nervous block de* In rder to test the possible c tribution by ent al eflex mechanism the effect f spinal sympathetic blockad pon the response t enous stasis as n est gated one subject A catheter was placed the epidural space bef o th measurements were started 1 expt ere c rried ut bef re and 3 after the epidural naesthesi had been duc d by marca ne (Henriksen nd Alsner 1975) The sweat test (Dhunér *t al* 1960) was performed bef re nd after the fusi n of marca ne, and afte the mea rements were term nated.

(*b*) *Local nervous blockade* The effect of local nervous blockade r lpha recepto block d upon th response t enous stasis was tud ed by ject g 1 ml of phentolamine (10 mg/ml) r 1 ml of Lidocain w thout asoconstri gent (20 mg/ml, 3 10 mol l) mixed with 0 2 ml Xenon isotonic sal ne int th a terio t bial muscle The experiments wer started 30 min later w th the subject placed n a supine position

( ) *Blockade f the as ular mooth muscle II* A passi ascula bed was nduced by ject ng 0.1 ml of h stamine as the base (1 mg/ml) m ed w th ^{133}Xenon to the a terior t bial muscle The activity w recorded every 5 s, and each period of measurement lasted o ly 1 5 m n

(*d*) *Measurement f v nous pr ssur* Venous press re wa measured d rectly o the dors m f the hand du i g en us tasis one subject placed i a supine position.

II *Chronic mpathetic denervat on*

1) Th experiments were performed on the brachioradial muscle n both arm of un laterally ympathecto mized patient, perated bout 9 years before The h nd n the side of operation as completely dry hile ther was t ll ma ual hyperhidrosi o the ther side (control side) The effect f enou tasis pon muscle blood flow was studied both arms.

) In bilaterally sympathect mized patient, t died t years postoperat el measurement ere perf rmed the brachioradial muscle, and the anterio t bial muscle wa used ontrol

C lculations and statistic

Mea perfu ion coefficient, f be calculated from the Kety-equat n f k λ 100 (ml 100 g mi) (K ty 1951), where k denotes the w shout rate co t t in min and λ the muscula tissue t blood part tion coefficient ml/g (0.7 ml/g) (Lassen *t l* 1964).

Relati blood flow $f_{(stasis)}/f_{(ref)}$ equals relati Xeno washout rat constant, $k_{(stasis)}$ $\bar{k}_{ref}$ the tissue t blood part tion coefficient, λ is assumed to be equal in the t o situation $\bar{k}_{ref}$ and $\bar{f}_{ref}$ her denote a rage alue of the washout rate constants (min) d the perfusion coefficients (ml/100 g mi) obtained just before nd after the test respecti ely

$k_{(stasis)}$ and $\bar{k}_{ref}$ were compared by means of the Student t test for paired samples The calculated relati e blood flow alues obtained during tasis and under the different cond tions were compared by means of the randomizatio test fo unpai ed samples. Level of sign ficance was chosen 0 05

Results

Blood flow decreased 30 per cent during enous stasis of 40 mmHg in all 4 subjects (Figs. 1 and 2)

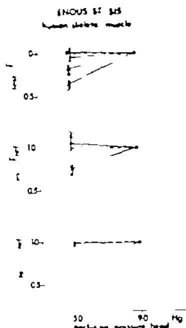


Fig. 1

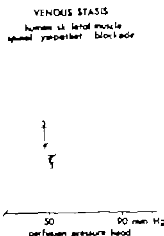


Fig. 2

Fig. 1 Relativ blood flo in human skeletal muscle during enous stasis of 40 mmHg obtained 3 normal subject plotted gainst perfusion pressure head (mmHg). f_{stasis} denotes perfusion coefficient obtained during venous stasis, and f_{rest} the erage perfusion coefficient calculated from the alues obtained just before and after the tes control, ● areas infiltrated ith phentolamine, areas infiltrated ith lidocaine, areas inf ltrated ith histamine Vertical lines ith bars denote 1 S E. Figures denote number of expts

Fig. Relativ blood flow in human skeletal muscle during enous stasis of 40 mmHg obtained before nd after spinal ympathetic blockade induced by epidural anesthesia, plotted gainst perfusion pressure head before blockade, ● after blockade Vertical lines ith bars denote 1 S E. Figures denote number of expts

The cal test became negative after the epidural anesthesia had been induced and was still negative after conclusion of the measurements, indicating that the sympathetic fibres had been blocked Arterial blood pressure emained almost constant during the measuring per d f_{rest} obtained just after the blockade was effective was approximately 50 per cent h gher than that obtained just before the blockade. Blood flow decreased 40% before (p 0.01) and 35 (p 0.05) after the blockade during enous stasis of 40 mmHg (Fig. 2). There was no difference in the response (p 0.7).

Lidocaine and phentolamine blocked the vasoconstrictor response to venous stasis as blood flow remained constant corresponding to decrease in ascular resistance (Fig. 1). When lidocaine (3 10 mol/l) was applied, relative blood flow S.E. was 0.97 0.05 4 p 0.6.

When the tissue was infiltrated with histamine, f_{rest} increased from 0.017 min⁻¹ to 0.56 min During venous stasis, blood flow in these areas decreased 16 per cent (p 0.001) Fig. 1). This response differed significantly f om that obtained in controls (p 0.001).

vascular smooth muscle cells, affecting myogenic reactivity Lidocaine (3 10 mol/l) did not affect myogenic activity in the rat portal vein, but blocked impulse transmission in sympathetic fibres effectively (Johansson and Ljung 1967). In this concentration lidocaine blocked the vasoconstrictor response effectively In two experiments phentolamine, 5 10 mol/l was applied. Even in this low concentration phentolamine blocked the vasoconstrictor response to venous stasis induced 15 min after the injection Four control expts. with injection of isotonic saline performed in order to test the effect of the injection trauma indicated that this trauma was without effect 15 min after the injection Phentolamine (5 10^{-8} mol/l) did not affect myogenic activity in the rat portal vein (Johansson and Mellander 1975).

The effect of lidocaine and phentolamine is, therefore, most likely due to a blockade of sympathetic adrenergic vasoconstrictor fibres, indicating that the vasoconstrictor response is due to a local nervous mechanism

Folkow and Öberg (1961) observed that venous stasis of 10 mmHg induced a decrease in blood flow in skeletal muscle in reserpinized cats, indicating that vascular reactivity is still present in more or less complete catecholamine depleted tissue The duration of the periods of measurement was less than 40 s. Jones and Berne (1964) observed a transient decrease in blood flow in the thigh muscle of dog during venous stasis of 70 mmHg, lasting about 40 s, whereafter blood flow almost returned to the level before the period of stasis. Lundvall and Mellander (1976) made similar observations in skeletal muscle of cat during external negative pressure of 40 mmHg. These observations suggest an intrinsic vascular response to increase in vascular transmural pressure

In humans a steady decrease in blood flow was observed during the venous stasis 5 days after sympathectomy this response was abolished, indicating that the vasoconstrictor response to venous stasis in humans rather is neurogenic than an intrinsic vascular response.

The findings in the reserpinized cats (Folkow and Öberg 1961) is in agreement with the observation that autoregulation in subcutaneous tissue was retained years after the sympathectomy (Henriksen 1976 b) This observation does not exclude an overlying nervous reinforcing mechanism.

When increase in venous transmural pressure during lowering of the limb by 40 cm was prevented by letting the subject tip his foot the vasoconstrictor response was blocked in subcutaneous tissue as vascular resistance increased only by 48 corresponding to the increase in arterial perfusion pressure head (autoregulation) (Henriksen and Sejrsen 1976 b). The abolishment of the vasconstrictor response during exercise might be due to liberation of metabolites in subcutaneous tissue affecting myogenic activity However elevation of venous transmural pressure during exercise by inducing venous stasis elicited an increase in vascular resistance of the same magnitude as in the lowered, resting leg. In patients with venous insufficiency exercise did not prevent the increase in venous transmural pressure and did not abolish the vasoconstrictor response Thus the abolishment of the vasoconstrictor response to lowering during exercise, is probably due to prevention of increase in venous transmural pressure, indicating that the vasoconstrictor response depends upon an impulse transmission from veins to arterioles. This furthermore strongly suggests that the vasoconstrictor response is due to a local nervous mechanism, probably a sympathetic axon reflex.

The findings in the present study are similar to those obtained in *human cutaneous* tissue and human subcutaneous adipose tissue (Henriksen and Sejrsen 1976 a, Henriksen Alsner 1975, Henriksen 1976 a, b, c, d). This means that blood flow in cutaneous tissue, subcutaneous adipose tissue, and skeletal muscle decreases about 50 % due to this local reflex mechanism, when enous transmural pressure is elevated about 25 mmHg or more.

Ph thysmography

Blood flow measured by enous plethysmography *might* be influenced by the reflex. With a cuff pressure of 40 mmHg it took between 40 and 60 s for the venous pressure to increase to the threshold level of the reflex, 25 mmHg, indicating that the reflex *is not elicited during* enous occlusion plethysmography for measurements of blood flow t low venous pressures. When the method is used for measurement of capillary filtration capacity (Mellander Öberg and Odelram 1964), possible invol ement of the reflex should be considered.

Haemodynamic implications of the refle

1) *Postural changes* from supine to upright position caused an erage decrease in cardiac output of 1 7 l/min (Hanson, Tabaken and Levy 1968), corresponding *to a decrease in total* ascular conductance of about 22 %. Assuming tha enous transmural pressure increases more than 25 mmHg in tissues corresponding to 50 of the b.w.t. and that the average decrease in blood flow is 2 ml/100 g min in these tissues, the local reflex mechanism would account for decrease in ascular conductance of about 10 %, which is 45 % of the total change. This means that about 45 of the postural changes in ascular tonus is due to this local reflex, occurring independently of the central nervous system. The remaining 55 are probably due to central reflex mechanisms elicited from baroreceptors located in the large arteries, central eins, and heart (Roddie and Shepherd 1957 Beiser *et al.* 1970 Rowell, Wyss and Brengelmann 1973)

) *Autoregulation of blood flow* (*Le* maintenance of constant blood flow during changes in arterial perfusion pressure head) became evident when the reflex was blocked, indicating that autoregulation is due to intrinsic mechanisms.

References

AMERY A. H. BOSSAERT M. DERUYTTERE, L. VANDERLINDEN and M. VERSTRAETE, Influence of body posture on leg blood flow. *Scand. J. clin. lab. Invest.* 1973 *31* Suppl 128. 29

BALDEN C. I. C. E. O. LUNDGREN, J. LUNDWALL and S. MELLANDER, Changes in elimination of 133Xenon from the *anterior tibial muscle* in man induced by immersion in ter and by shift in body position *Aerospace Med* 1971 4 489 493

BEISER, G. D. R. ZELIS, S. E. EPSTEIN, D. T. MASON and E. BRAUNWALD, The Role of Skin and Muscle Resistance Vessels in Reflexes Mediated by the Baroreceptor System. *J. clin. Invest.* 1970 49 225–231

COBBOLD A. B. F. ow. I KJELLMER and S. MELLANDER, Nervous and local chemical control of pre capillary sphincters in skeletal muscle as measured by changes in filtration coefficient. *Acta physiol. scand.* 196 5 180-19

ONN H. L. Jr. Equilibrium distribution of radioxenon in tissue: Xenon-hemoglobin association curve. *J. appl. Physiol.* 196 16 1065 1070

DUNCKER, K. G. S. LINDQUIST and A. WILLIAMS, Ninhydrin Test — an objective method for testing local anaesthetic drugs. *Acta anaesth. scand.* 1960 4 189–198.

Folkow, B. and B. Öberg, Autoregulation and basal tone in consecutive vascular sections of the muscles in reserpine-treated cats. *Acta physiol. scand.* 1961. 53. 105–113.

Hanson, J. S., B. S. Tabakin and A. M. Levy, Comparative exercise-cardio-respiratory per… of normal men in the third, fourth, and fifth decades of life. *Circulation* 1968. 37. 345–360.

Henriksen, O. Effect of somaesthetic denervation upon the vasoconstrictor response in hu… cutaneous tissue. *Acta physiol. scand.* 1976 a. 96. 431–432.

Henriksen, O. Effect of chronic sympathetic denervation upon blood flow in human subcutane… *Acta physiol. scand.* 1976 b. 97. 377–384.

Henriksen, O. Local nervous mechanism in regulation of blood flow in human subcutaneo… *Acta physiol. scand.* 1976 c. 97. 385–391.

Henriksen, O. Local reflex in microcirculation in human subcutaneous tissue. *Acta physi…* 1976 d. 97. 447–456.

Henriksen, O. and T. Alsner, Effect of spinal sympathetic blockade upon local regulation of b… in subcutaneous tissue. *Acta physiol. scand.* 1975. 95. 83–88.

Henriksen, O. and P. Sejrsen, Local reflex in microcirculation in human cutaneous tissue. *Act… scand.* 1976 a. 98. 227–231.

Henriksen, O. and P. Sejrsen, Effect of "veinpump" activation upon venous pressure and bloo… human subcutaneous tissue. *Acta physiol. scand.* 1976 b. Suppl. 440. Abstract No. 197. p. 129.

Henriksen, O., S. L. Nielsen, W. P. Paaske and P. Sejrsen, Autoregulation of blood flow … cutaneous tissue. *Acta physiol. scand.* 1973. 89. 538–543.

Johansson, B. and B. Ljung, Spread of excitation in the smooth muscle of the rat portal vein. *Act… scand.* 1967. 70. 312–322.

Johansson, B., B. Ljung, T. Malmfors and L. Olson, Denervation supersensitivity in the … innervated smooth muscle of the portal vein. *Acta physiol. scand.* 1969. Suppl. 330. 58.

Johansson, B. and S. Mellander, Static and dynamic components in vascular myogenic res… passive changes in length as revealed by electrical and mechanical recordings from the rat po… *Circulat. Res.* 1975. 36. 76–83.

Jones, R. D. and R. M. Berne, Local regulation of blood flow in skeletal muscle. *Circulat. R…* 15. Suppl. I. 30–38.

Lassen, N. A., I. F. Lindbjerg and O. Munck, Measurement of blood flow through skeletal m… intramuscular injection of Xenon-133. *Lancet* 1964. I. 686.

Lundvall, J. and S. Mellander, 1976. Personal communication.

Mellander, S., B. Öberg and H. Odelram, Vascular adjustments to increased transmural pr… cat and man with special reference to shifts in capillary fluid transfer. *Acta physiol. scand.* 1964. 6…

Roddie, I. C. and J. T. Shepherd, The effects of carotid artery compression in man with spe… erence to changes in vascular resistance in the limbs. *J. Physiol.* (Lond.) 1957. 139. 377–384.

Rowell, L. B., C. R. Wyss and G. L. Brengelmann, Sustained human skin and muscle vasocon… with reduced baroreceptor activity. *J. appl. Physiol.* 1973. 3. 639–643.

Sejrsen, P. and K. H. Tønnesen, Shunting by diffusion of inert gas in skeletal muscle. *Acta physiol. sc…* 1972. 86. 82–91.

Tønnesen, K. H. and P. Sejrsen, Inert gas diffusion method for measurement of blood flow. Com… of bolus injection to directly measured blood flow in isolated gastrocnemius muscle. *Circul…* 1967. 20. 552–564.

Tønnesen, K. H. and P. Sejrsen, Washout of [133]Xenon after intramuscular injection and direct m… ment of blood flow in skeletal muscle. *Scand. J. clin. Lab. Invest.* 1970. 25. 71–81.

Acta physiol scand. 1977 99 27–36

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Efflux of 5-Hydroxytryptamine from Synaptosomes of Rat Cerebral Cortex

By

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Abstract

ROSS, S. B. and D. KELDER. *Efflux of 5-hydroxytryptamine from synaptosomes of rat cerebral cortex.* Acta physiol. scand. 1977 99 27–36.

The efflux of ^{3}H-5-HT from a crude synaptosome preparation of the cerebral cortex of reserpinised rats was examined. The synaptosomes were loaded with ^{3}H-5-HT by pre-incubation of the homogenate in presence of pargyline and desipramine in order to inhibit deamination of 5-HT and uptake into noradrenergic neurones. The synaptosomes were collected by centrifugation, washed and resuspended in 0.25 M sucrose. No spontaneous efflux of 5-HT was detectable at 0°C but marked efflux was observed at 27°C and 37°C. 4-Chloroamphetamine, low external Na^{+} concentration, ouabain and the depolarizing agent veratridine markedly accelerated the initial (5 min) efflux. Inhibitors of the neuronal 5-HT uptake, *e.g.* chlorimipramine, H 102/09 and A 23189 antagonized the 5-HT efflux evoked by these means, whereas desipramine, which is a poor inhibitor of the 5-HT uptake, had only slight effect on the 5-HT efflux. It is suggested that 5-HT can be actively transported out from the synaptosomes by the reversed 5-HT uptake mechanism.

Key words: 5-Hydroxytryptamine efflux, 4-chloroamphetamine, chlorimipramine, H 102/09, A 23189

The physiological importance of the neuronal membranal uptake mechanisms for the biogenic monoamines is generally recognized and accepted. Due to the hydrophilic nature of these amines they slowly pass the neurone membranes by diffusion and active uptake mechanisms are therefore necessary for a rapid transport of these amines through the membranes. It is also a general accepted view that the indirectly acting sympathomimetic amines exert their action by releasing the biogenic monoamines from intraneuronal binding sites (Trendelenburg 1972). From pharmacological observations it has been concluded that this release is different from that produced by nerve stimulation (Furchgott *et al.* 1963). Recently Thoa *et al.* (1975) found that the release of noradrenaline by the sympathomimetic amines in contrast to that of nerve stimulation in the isolated vas deferens was not joined by release of the enzyme dopamine-β-hydroxylase. If the release of the biogenic amines does not occur with an exocytosis mechanism the problem remains how the released amines pass the neurone membranes outwardly since the same barrier exists in both directions. Until recently this problem has been overlooked, although it was suggested as possible tha

TABLE I Inhibition of the efflux of H 5-HT from synaptosomes evoked by 4-chloroamphetamine (5) M), low external Na (26 mM NaCl replaced by 250 mM sucrose) and by veratridine (5 10^{-5}) The cortical synaptosomes were loaded with H 5-HT (1 10^{-} M) in presence of 2.4 10^{-} pargyline and 3 10^{-} M desipramine for 20 min The efflux of H 5-HT was determined incubating 100 / l of the washed suspension in 2.0 ml Krebs buffer or modified buffer for 5 at 37°C. The difference in the amount of radioactivity in the pellet in absence and presence the efflux inducer was taken as the evoked efflux. The inhibition of the evoked efflux was calculated in per cent Each value is the mean ± S.E. of 4 determinations.

Compound	Conc. M	% inhibition 4-Chloro-amphetamine	Low Na	Veratridine
H 102/09	2.5 10^{-}	14±2	—	—
	2.5 10^{-}	48±2	61±6	59±6
A 23189	3 4 10^{-}	48±4	60±14	69±5
Chlorimipramine	2.9 10^{-8}	—	56±11	43±3
	2.9 10^{-}	79±3	89±5	63±3
Desipramine	3.0 10^{-}	1 ±3	70±5	19±4
	3 0 10^{-4}	—	9±3	40±5
Millicaine	2.9 10^{-4}	—	8±4	82±3

0 05 > P > 0.01 0.01 > P > 0 001 P 0.001 (Student's t test).

with the exception that H 5-HT was omitted in the pre-incubation of the homogenat The uptake of H 5-HT for a period of 5 min was examined at times corresponding to th start of the efflux experiment and after 60 min incubation at 37°C. The accumulation de creased from 64 2 ± 9 0 (n 5) to 16.8 ± 1 8 (n = 5) pmol/g tissue/min incubation *i.e.* th uptake after 60 min incubation was only 6 % of that at zero time. Thus, the partial purified synaptosomes had lost a large part of their capacity to accumulate H 5-HT t the incubation

Effect of uptake inhibitors on the efflux of H 5-HT

The effect of H 102/09 a selective inhibitor of the membranal 5-HT uptake (Ross *et a* 1976), on the efflux of H 5-HT from the synaptosomes at 37°C was examined (Fig. 1 A H 102/09 itself caused an increase of the spontaneous efflux, which was more marked afte prolonged incubation. However it significantly inhibited the initial increase of the efflu produced by 4-chloroamphetamine, by low external Na and by veratridine (Table I) Chlorimipramine (Shaskan and Snyder 1970) and A 23189 (unpublished observations are two other potent inhibitors of the 5-HT uptake. As shown in Table I these compound inhibited the efflux of H 5-HT induced by the various means examined. Desipramine which *is a potent inhibitor of the NA uptake but a rather poor inhibitor of the 5-HT uptake* (Ross and Renyi 1975) had much less effect in inhibiting the 5-HT efflux. At the higher concentration of desipramine examined the effect on the veratridine induced efflux was larger than on that evoked by low external Na The sam discrimination between the efflux evoked by veratridine and low external Na was ol the local anesthetic agent millicaine (Table I).

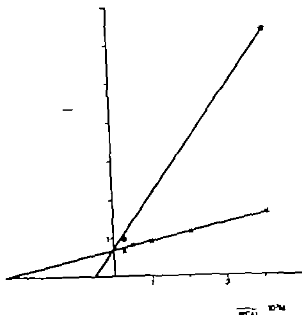


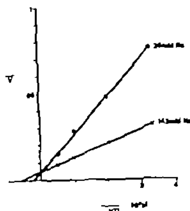
Fig. 2 Double reciprocal plot of the increase of the ³H-5-HT efflux caused by 4-chloroamphetamine (PCA). Synaptosomes were prepared and loaded with ³H 5-HT as described in the legend of Fig. 1. The incubation time was 5 min. The velocity of efflux ( ) was determined as the difference of the efflux at 37°C and 0 C and was expressed as pmol/g/min. The concentration of H 102/09 (●) was 2.5 10^{-7} M. Each value is the mean of 4 determinations.

Kinetics of the antagonism of H 102/09 on the 4-chloroamphetamine induced increase of ³H-5-HT efflux

The kinetics of the antagonism of H 102/09 on the increased ³H-5-HT efflux produced by 4-chloroamphetamine was examined according to the method of Lineweaver and Burk (1934). The increased initial (5 min) efflux caused by 5 different concentrations of 4-chloroamphetamine (.4 10 M 3.9 10 M) were determined in absence or presence of H 102/09 (.5 10 M). The result of an experiment is shown in Fig. 2. H 102/09 inhibited competitively the effect of 4-chloroamphetamine. The apparent K_m for 4-chloroamphetamine in increasing the efflux was 4.6 10^{-6} (mean of 2 determinations. 5.4 10^{-6} M and 3.7 10 M). The inhibitor constant (K_i) for H 102/09 was 1.1 10^{-} M (mean of 1.5 10^{-} M and 6.1 10 M). Both these values are close to the corresponding K_i values for the inhibition of the 5-HT uptake in the same synaptosome preparation being 1.2 10^{-} M for 4-chloroamphetamine and 1.8 10 M for H 102/09 (Fig. 3). The apparent K_m value for 5-HT was in these experiments 8 10 M.

Effect of ouabain on the increase in efflux produced by 4-chloroamphetamine

It was of interest to examine, if ouabain antagonized the increase in efflux produced by 4-chloroamphetamine, since if so, it should indicate that 4-chloroamphetamine is actively transported by a Na dependent mechanism into the synaptosomes before it releases 5-HT



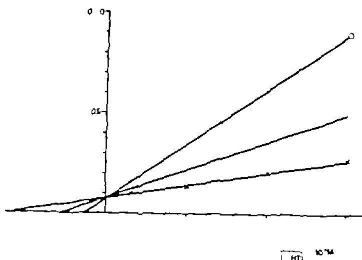
[illegible] 4 Double reciprocal plot of the uptake of 5-HT in [illegible] of rat cerebral cortex (non-reserpinized) at [illegible] different Na^+ concentrations. The incubation time was [illegible] min. The uptake velocity (V) was determined as described [in] the legend of Fig. 3

[ex]ternal Na^+ concentration, whereas V_{max} was unchanged. The apparent K_m value for [n]ormal Na^+ concentration (1.7 × 10⁻ M) was in this experiment twice higher than that [o]btained with the more purified synaptosomes obtained from reserpinized rats.

Discussion

The marked spontaneous efflux of ³H-5-HT from the cerebral cortical synaptosomes observed in this study is in accordance with the findings by Bogdanski *et al.* (1968). Since it was temperature dependent it is probably due to metabolic processes. Bogdanski *et al.* (1968) found that the labelled 5-HT decreased more rapidly than the endogenous 5-HT in the synaptosomes and proposed that dilution of the labelled amine with newly synthesized 5-HT [w]as one reason for the decline in the labelled 5-HT. No attempt to test this hypothesis with an inhibitor of the 5-HT synthesis has been performed. Another possible mechanism is that the synaptosomes are unstable under the incubation conditions employed, are broken and release their contents out to the medium. The loss of a large part of the capacity of the synaptosomes to accumulate 5-HT after 60 min incubation at 37 C indicates that a part of the synaptosomes deteriorate in some way. This may at least partially contribute to the [r]apid spontaneous efflux of 5-HT observed at this temperature.

In spite of this spontaneous efflux of 5-HT very constant and reliable values were obtained [w]hen determining the initial 5 min efflux evoked by 4-chloroamphetamine, low external Na^+, ouabain and [t]eratridine. Since this evoked 5-HT efflux was temperature dependent and [wa]s antagonized by uptake inhibitors it is probably an active outward transport of 5-HT. The observation that H 102/09 which is a selective inhibitor of the neuronal 5-HT uptake (Ross *et al.* 1976), accelerated the 5-HT efflux but antagonized the efflux evoked by [v]arious mean[s] appears somewhat conflicting. The acceleration of the efflux is understood, [i]f it is supposed that a part of 5-HT released is taken up again into the synaptosomes by the membranal transport mechanism. The finding that the effect of H 102/09 increased [w]ith time is in accordance with this interpretation, since the medium concentration of 5-HT increased and accordingly the uptake velocity. The antagonism of the evoked efflux is,



F g 3 Double reciprocal plot of the uptake f H 5-HT cerebr l cortical synaptosomes from experi at The ncubatio t me w 5 m Th uptake elocity ( ) was determined a the difference at 37 C 0 C nd was p essed i pmol g mi The concent atio f H 10 '09 ( ) was 5 10 M and the 4-chloroamphetamin ( ) 5 10 M Each alue is the mean of 4 det rminations

intraneuronally The synaptosomes were simultaneously incubated with ouabain u medium free of K and 4-chloroamphetamine at low concentration (1 10 M) in o to be within an adequate concentration range for the potential uptake (Table II) The ef of this combination was larger than that of 4-chloroamphetamine and ouabain alo which indicates an additive effect

Kinetic determination of the Na dependence of the 5-HT transport

Since both influx and efflux of 5-HT in synaptosomes are influenced by Na (Bogdan *et al* 1968) it was of interest to examine kinetically the nature of the Na dependen This was best performed by working with the uptake system, since the 5-HT concentrati can then be properly controlled In this experiment we used non reserpinized rats and crude cortical homogenate obtained after low speed centrifugation (800 *g*) The determi tion of the uptake of H 5-HT (3 10 M 2 10 M) by the synaptosomes in the hom genate was performed in normal buffer and in buffer low in Na (26 mM) by replacing Na with 117 mM LiCl in order to keep the Cl concentration unchanged The double recipro plots (Fig. 4) show that the affinity of 5-HT for the uptake sites was decreased by the l

TABLE II The combined effect of uaba (5 10 M) no external K d 4-chloroamphetam (1 10 M) on the efflux of H 5-HT from cerebr l cortical synaptosomes of the rat The cubation was perf rmed for 5 min Each lue s mean S E of 8 determ nat ons

	ΔEfflux pmol g min S E	p
1 Ouabai 0 K	5 0 0 3	
2. 4-Chloroamphet mine	10 2 0 3	
3 4-Chloroamphetamine ouabai 0 K	12 8 0 1	0 001 (3)

Student t test

n active transport of 4-chloroamphetamine into the synaptosomes and thereby preventing the intraneuronal release of ³H-5-HT. However, the finding that ouabain did not inhibit the effect of 4-chloroamphetamine but instead added its own effect to that of 4-chloroamphetamine indicates that 4-chloroamphetamine was not transported by the membranal 5-HT transfer system. Experiments with purified synaptosomes have also shown that ³H-4-chloroamphetamine has high affinity for the synaptosomes but no active, Na⁺-dependent accumulation was observed (Ross 1976 b).

The inhibition by 4-chloroamphetamine of the 5-HT uptake in the synaptosomes may not in fact be due to a real inhibition of the uptake but due to its 5-HT releasing effect. If the hypothesis of a reversible transport mechanism is correct the influx of 5-HT should equilibrate with the efflux at a much lower ratio between the inner and outer concentration of 5-HT than under normal condition, if the extravesicular binding sites are occupied by 4-chloroamphetamine, which results in an elevated concentration of the free concentration of 5-HT. This hypothetical effect of 4-chloroamphetamine should accordingly be very similar to an inhibition of the uptake although the primary attack is not located to the transfer sites. This suggestion is in accordance with the observation that 4-chloroamphetamine was more or at least as potent in increasing the efflux than in inhibiting the uptake of 5-HT. According to the kinetic experiments H 102/09 inhibited competitively the increased efflux produced by 4-chloroamphetamine. Since the release by 4-chloroamphetamine of intraneuronally bound ³H-5-HT was probably directly related to the concentration of 4-chloroamphetamine the observed competitive nature of the effect of H 102/09 is not contradictory to the hypothesis that H 102/09 inhibited the outward transport of 5-HT. The almost identical K_i values for H 102/09 in inhibiting the 5-HT uptake and the 4-chloroamphetamine induced 5-HT efflux further support the hypothesis of an identical mechanism for the uptake of 5-HT and the efflux of 5-HT evoked by 4-chloroamphetamine.

Alternative explanations of the data are possible. For instance, it can not be excluded that the influx and efflux are two separate mechanisms and that the inhibitors of the 5-HT uptake also antagonized the efflux mechanism due to similar structure requirements for both mechanisms.

References

BLASZKOWSKI, T. P. and D. F. BOGDANSKI, Evidence for sodium dependent outward transport of ³H-norepinephrine mediated by calcium at the adrenergic synapse. Inhibition of transport by desipramine. *Life Sci.* 1972, *11*, 867–876.

BOGDANSKI, D. F., A. TISSARI and B. B. BRODIE, Role of sodium, potassium, ouabain and reserpine in uptake, storage and metabolism of biogenic amines in synaptosomes. *Life Sci.* 1968, *7*, 419–428.

FULLER, R. W., C. W. HINES and J. MILLS, Lowering of brain serotonin level by chloroamphetamines. *Biochem. Pharmacol.* 1965, *14*, 483–488.

FURCHGOTT, R. F., S. M. KIRPEKAR, M. RIEKER and A. SCHWAB, Actions and interactions of norepinephrine, tyramine and cocaine on aortic strips of rabbit and left atria of guinea-pig and cat. *J. Pharmacol. exp. Ther.* 1963, *142*, 39–58.

HOLZ, R. W. and J. T. COYLE, The effects of various salts, temperature, and the alkaloids veratridine and batrachotoxin on the uptake of [³H]-dopamine into synaptosomes from rat striatum. *Molecul. Pharmacol.* 1974, *10*, 746–758.

LINEWEAVER, H. and D. BURK, The determination of enzyme dissociation constants. *J. Amer. Chem. Soc.* 1934, *56*, 658–666.

on the other hand understood if the transport of 5-HT under these experimental conditions occurs with the reversed uptake mechanism and this efflux accordingly inhibited by the uptake inhibitors.

There are several observations supporting the hypothesis that the membranal 5-HT transport mechanism can be reversed. The Na dependence of the influx and efflux of 5-HT (Bogdanski *et al* 1968) indicates this possibility since several other Na dependent transport mechanisms are known to be reversible (Schultz and Curran 1970) Na influences these transports either by increasing the affinity of the solute for the uptake sites or by increasing the velocity of the transport. Observation in the present study indicates that the affinity of 5-HT is increased by Na which is in variance with the transport systems for noradrenaline (Sugrue and Shore 1971) and dopamine (Holz and Coyle 1974) in which the velocity of the transport but not the affinity appears to be increased. The explanation of the efflux of 5-HT by low external Na may accordingly be due to an equalization of the affinities on the two sides of the neurone membrane, which because of the much larger 5-HT concentration inside the synaptosomes results in the outward transport Ouabain by inhibiting the Na pump increase the Na concentration in the synaptosomes and thereby the affinity of the 5-HT for the carrier sites on the inside of the membrane. Veratridine increases the Na permeability of excitable membranes (Ulbricht 1969) with the same result as with ouabain but with a more rapid onset of action. The finding that the local anesthetic agent millicaine discriminated between the 5-HT efflux induced by veratridine and low external Na indicates that the membrane stabilizing effect of the test compounds was not involved in the inhibition of the 5-HT efflux mechanism. Thus, the effect by millicaine on the veratridine evoked efflux could be explained by the antagonism of the depolarizing effect whereas the corresponding effect by the uptake inhibitors was probably due to the inhibition of the 5-HT transport. In accordance with this view a comparatively high concentration of desipramine, which is a rather poor inhibitor of the 5-HT uptake (Ross and Renyi 1975), had large effect on the veratridine evoked efflux, reflecting the membrane stabilizing action at this concentration. Indeed inhibition of the release of neurotransmitters induced by veratridine in synaptosomal preparations may become a valuable method for testing drugs on the membrane stabilizing action under identical or similar *in vitro* conditions as used in other assays. However the test compound must not inhibit the active outward transport of the transmitter examined

The efflux induced by 4-chloroamphetamine can also be explained by the hypothesis of the reversed transport mechanism. This amine has high affinity for 5-HT neurones (Pletscher *et al* 1964 Fuller *et al* 1965). Since the vesicular storage mechanism of 5-HT was destroyed by reserpine, 4-chloroamphetamine, which because of its large lipophilicity readily passes membrane barriers, probably released H 5-HT bound to some extravesicular binding sites in the nerve endings. The rise in the concentration of the soluble fraction of 5-HT in the synaptomes results in increase in the outward transport of 5-HT since the rate of the transport is directly related to the concentration of 5-HT within the ranges of the transport capacity The inhibition by the 5-HT uptake inhibitors of the efflux evoked by 4-chloroamphetamine, low external Na and high internal Na at the same concentration ranges support this hypothesis. It can be argued that the uptake inhibitors antagonized

...n active transport of 4-chloroamphetamine into the synaptosomes and thereby preventing ...e intraneuronal release of H-5-HT. However the finding that ouabain did not inhibit ...e effect of 4-chloroamphetamine but instead added its own effect to that of 4-chloro-...amphetamine indicates that 4-chloroamphetamine was not transported by the membranal ...5-HT transfer system. Experiments with purified synaptosomes have also shown that H ...-chloroamphetamine has high affinity for the synaptosomes but no active, Na dependent ...accumulation was observed (Ross 1976 b).

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References

BLAUSTEIN... T. P. and D. P. BOGDANSKI, E...idence for sodium dependent outward transport of the ³H-norepinephrine mobilized by calcium at the adrenergic synapse. Inhibition of transport by desipramine. *Life Sci.* 1977 *11* 867–876.

BOGDANSKI, D. F., A. TISSARI and B. B. BRODIE, Role of sodium, potassium, ouabain and reserpine in uptake, storage and metabolism of biogenic amines in synaptosomes. *Life Sci.* 1968 *7* 419–428.

FULLER, R. W., C. W. HINES and J. MILLS, Lowering of brain serotonin level by chloroamphetamines. *Biochem. Pharmacol.* 1965 *14* 483–488.

F...HMAN... R. F., S. M. KIRPEKAR, M. RIKLE... and A. SVENSSON, Actions and interactions of norepinephrine, tyramine and cocaine on aortic strips of rabbit and left atria of guinea-pig and cat. *J. Pharmacol. Exp. Ther.* 1963 *142* 39–58.

H... R. W. and J. T. C... The effects of ...anion salts, temperature, and the alkaloids ...coutndine and ...ucholetoun on the uptake of [³H]-dopamine into ...ynaptosomes from rat striatum. *Molecul. Pharmacol.* 19... *12* 746–758.

L... ...H. and D. B...k, The determination of enzyme dissociation constants. *J. Amer. Chem. Soc.* 19... 56 658–666.

PATON, D M Mechanism of efflux of noradrenaline from adrenergic nerves in rabbit tru. *Br Pharmacol.* 1973 *49* 614–627

PLETSCHER, A., G BARTHOLINI, H. BRUDERER, W P BURKARD and K. F GEY Chlorinated arylalkylam affecting the cerebral metabolism of 5-hydroxytryptamine. *J Pharmacol. exp. Ther* 1964. *145* 344-35

ROSS, S. B., Structural requirements for uptake into catecholamine neurones. In: *The mechanisms of neur. and extraneuronal transport of catecholamines.* Ed. D M Paton, 1976 a, pp 67 93, Raven Press N York

ROSS, S. B., Antagonism of the acute and long-term biochemical effects of 4-chloramphetamine on t 5-HT neurones i the rat brain by inh bit rs of the 5-hydroxytryptamine uptake. *Acta pharmacol.* (Kb 1976 b. *39* 456–476.

ROSS, S. B. nd A. L. Renyi, Inh bition of the uptake of tritiated 5-hydro ytryptamine in brain tis *Europ J Pharmacol.* 1969 *7* 270–277

ROSS, S. B. and A L. RENYI, Uptake and metabolism of β-phenylethylamine and tyramine in mouse br and heart slices. *J Pharm Pharmacol* 1971 *23* 276–279

ROSS, S. B. and A. L. RENYI Tricyclic antidepressant agents. I Comparison of the uptake of H-noradre line and [14]C 5-hydroxytryptamine in sl ces and crude synaptosome preparations of the midbrain-hy thalamus region of the rat brain. *A ta pharmacol* (Kbh.) 1975. *36* 382 394.

ROSS, S. B., A. L. RENYI a d B. BRUNFELTER, Cocaine-sensitive uptake of sympathomimetic mines nerve tissue. *J Pharm Pharmacol* 1968 *20.* 283–288

ROSS, S. B. and D KELDER, Active transport of H-bretyl um in the rat vas deferens in v tro. *Acta phys scand* 1976. *97* 209–221

ROSS, S. B., S.-O ÖGREN and A. L. RENYI, (Z)-Dimethylamino-1-(4-bromophenyl)-1-(3-pyridyl)prop (H 102/09) and new selective inhibito of the neuronal 5-hydro ytryptamine uptake. *A ta pharma* (Kbh) 1976. *39* 152 166.

SCHULTZ, S G and P F CURRAN, Coupled transport of sodi m and organic solutes. *Ph. siol Rev* 19 *50* 637 718

SHASKAN E and S. H SNYDER, Kinetics of H 5-hydro ytryptamine uptake to ynaptosomes in different regions f the brain *J Pharmacol xp Ther* 1970 *175* 404–418

SNYDER, S. H and J T COYLE, Regional differences in H[3]-norepinephrine and H[3]-dopamine uptake a rat brain homogenates. *J Pharmacol p. Ther* 1969 *165* 78–86.

SUGRUE, M F and P A SHORE, Further evidence for sodium-dependent optically specific and reserpi sensitive amine carrier mechanism at the adrenergic neuron. *J Pharmacol xp Ther* 1971 *177* 389-39

THOA, N B., G F WOOTEN, J AXELROD and I J KOPIN On the mechanism of release of orepineph from sympathetic nerves induced by depolarizing agents and sympathomimetic drugs. *Mol. Pharmac* 1975 *11* 10–18.

TRENDELENBURG, U Classification of ympathomimetic amines. In *Catecholamines.* Ed. H Blasch and E. Muscholl, 1972, pp 336–36_. Springer Verlag Berlin, Heidelberg, New York.

ULBRICHT W The effect of eratridine on excitable membranes of nerve and muscle. *Ergebn Phys* 1969 *61* 18–71

Acta physiol. scand. 1977. 99. 37–41

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Preferential Uptake of [14]C-α Aminoisobutyric Acid into Mouse Uterine Tissue during Early Pregnancy

By

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Abstract

LINDQVIST L., O. NILSSON and G. RONQUIST *Preferential uptake of C α-aminoisobutyric acid into mouse uterine tissue during early pregnancy* Acta physiol. scand. 1977. 99. 37–41

The uptake of ^{14}C-AIB into uterine tissue and some other organs was studied during early implantation. Mice in experimentally delayed implantation were given estrogen and ^{14}C AIB at different time intervals. Mice in delayed implantation given only ^{14}C-AIB displayed low transport rate into the uterine tissue comparable ith that for the diaphragm muscle. However, if the estrogen injection preceded that of th ^{14}C AIB by 4 to 8 h the transport capacity increased by factor of at least 4 times hile the uptake for th diaphragm muscle remained low. Under such conditions ^{14}C-AIB-accumulation into uterine tissue wa favoured for at least 4 h of in vitro incubation ith the labeled amino acid.

The rate of amino acid transport appears to be closely correlated to the rate of protei synthesis of both normal and malignant tissues (Riggs 1964, Griffiths 1967). Glucocorticoid hich are catabolic hormones, work antagonistically to the transport of amino acids int skeletal muscle (Riggs 1964, Christensen 1975), while insulin and growth hormone, fo example, which are both anabolic hormones and promote protein synthesis accelera amino acid transport into several tissues (Riggs 1964). Also estrogens promote growth particular tissues (Noall *et al.* 1957. Noall and Allen 1961) and consequently McCorquoda and Mueller (1958) observed an increased activity of amino acid-activating enzyme in t uterus already 3 h after treatment with estrogen. An early "induced protein after exposu of at uterus to estradiol, has recently been detected among the soluble proteins (Pennequ Robel and Baulieu 1975).

The capacity to transport amino acids is probably also higher since the level of free ami acid is higher in the more rapidly growing tissues (Noall *et al.* 1958). This is tested by us α-aminoisobutyric acid, AIB, as an *amino acid substrate* since this amino acid is not me bolized. As expected, Noall *et al.* (1958) found a 3-fold increase in the uterine accumulat of AIB upon administration of estradiol, and Riggs and Pan (1972) observed an increa

in vitro transport of AIB into the estrogen-primed uterus. Other studies also indicate influence *in vitro* by estrogen on AIB uptake and transport (Riggs *et al* 1968, Walters *et* 1975)

Since early pregnancy specifically the time of blastocyst implantation, is associated w an increase in the concentration of estrogen we questioned whether the capacity of A transport is increased as well If so this might have implications for the nutrition of t blastocyst at trophoblast attachment and invasion Therefore, the aim of the present inve gation was, firstly to examine the characteristics of AIB transport *in vivo* into some org of mice, conditioned for experimentally delayed implantation (Humphrey 1967), an secondly to examine whether the capacity of AIB transport *in vivo* into the uterus and so other organs increased after the initiation of implantation by estrogen.

Materials and methods

^{14}C-α-aminoisobutyric acid (1 ^{14}C AIB), Protosol and Aquasol were purchased from NEN Chem GmbH Dreieichenhain, W Germany and α aminoisobutyric acid from Calbiochem, San Diego, US Progesteron was purchased from Ikapharm, Ramat-Gan, Israel and estradiol from AB Leo, Hälsingbo Sweden. All chemicals used were analytical grade. Radioactivity was measured in a Nuclear Chica Uniflux II Liquid Scintillation Counter

Adult white female mice (N M R.I. N al Medical Res Inst Bethesda, USA) were mated and the d the vaginal plug was seen was recorded as day 1 They were spayed day 3 and given the first dose of mg f progesteron i 0.04 ml pean t oil subcutaneously the same day The animals were kept in dela implantation by daily injections f progresteron. Impla tation was initiated by a subcutaneous injectio of 0.1 g estradiol-17B i 0.1 ml propyleneglycol. Generally 4–5 μCi ^{14}C AIB in 0.1 ml of a 40 mM A solution, made isotonic with NaCl, was given i in the tail to each animal Blood samples ere dra f m the cut tail and directly transferred to the liquid scintillatio bottles. Tissue samples ere washed o n ice-cold Krebs-Ringer bicarbonat buffe freshly bubbled with a gas mi ture of 93 5 O_2 and 6.5 CO_2 to give a pH of 7 4 and l ghtly blotted on tissue paper before being transferred t the bottles. T animals were sacrificed by giving them Nembutal before the tissue samples were taken

Samples were transferred into weighed liquid scintillatio counting bottles, weighed and dissolved ov night i 1 ml Protosol at 50°C. The solutions were mixed w th 50 μl conc. acetic acid (to minimize "lig sensitivity") and 10 ml of Aquasol. Samples were then counted and countings corrected for self absorptio

Results

The clearance of ^{14}C AIB was established by determining the time at which the amin acid concentration curve of the blood crossed that of the urine. The labeled amino acid wa i.v administered to the animals which were then sacrificed at different times betwe 5 and 20 min after the injection of the labeled amino acid Fig. 1 shows that a peak value blood was reached after 15 min and that the declining blood curve crossed the rising cur reflecting the urine concentration of ^{14}C AIB after 20 min The curve profiles of both bloo and urine were quite similar in 4 different expts. with animals in delayed implantation well as with estrogen-treated animals. It was therefore convenient to sacrifice the anima 20 min after the i v administration of ^{14}C AIB during the next series of expts.

The distribution of ^{14}C AIB into the uterus and some other tissues during early implanta tion was examined in 4 groups, each containing 3 mice in experimentally delayed implanta tion Animals of one group (controls) were given only ^{14}C AIB 20 min before death. Th

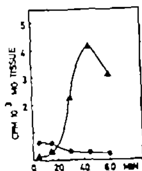


Fig. 1

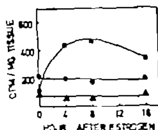


Fig. 2

Fig. 1. ¹⁴C AIB concentration in blood and recovery in uterus at different times after intravenous administration ● — ● blood, ▲ — ▲ uterus

Fig. Recovery of ¹⁴C AIB in blood (●—●), diaphragm muscle (▲—) and uterine tissue (■—) at different stages of implantation initiated by estrogen. Stand. dev. max ± 40 cpm.

mice of the other 3 groups were given an injection of estrogen, and ¹⁴C-AIB was administered 20 min before death, 4, 8 and 16 h respectively after the estrogen injection. Blood, diaphragm muscle and uterus were examined.

The level of ¹⁴C AIB in the blood was about the same between 0 and 16 h after the injection of estrogen. The *in vivo* uptake by the diaphragm muscle was quite low and independent of the time after the estrogen injection. The *in vivo* uptake by the uterine tissue, on the contrary displayed a characteristic profile with a low uptake of ¹⁴C-AIB for animals in delayed implantation (controls), marked increase at 4 and 8 h after the initiation of implantation by estrogen and a slight decrease at 16 h as compared with the values for 4 and 8 h (Fig. 2).

The retention of ¹⁴C AIB was measured in blood, diaphragm muscle, brain and uterus. Mice were given the labeled amino acid 4 h after the injection of estrogen and the tissue concentrations were measured after different *in vivo* incubation times with the labeled amino acid substrate 5 groups, each containing at least 3 animals in experimentally delayed implantation were used The animals were sacrificed 1/3, 4, 16, 24 and 48 h after the injection of ¹⁴C AIB (Fig. 3)

Although the blood concentration of the labeled amino acid was declining already at 20 min after the administration (cf Fig. 1) and remained low throughout the expt., the ¹⁴C AIB was retained in the uterus for several hours. Still at 16 h the uterine concentration of the labeled amino acid was higher than the 20 min blood value. Also after 16 h the blood value was 7 times lower than that of the uterus. Diaphragm muscle showed a slow accumulation of ¹⁴C AIB during at least the first 4 h and retained the labeled amino acid for several hours The brain displayed a somewhat longer accumulation time and a higher uptake than the diaphragm muscle The uptake of ¹⁴C AIB was nevertheless much lower in these tissues than in the uterus The prolonged uptake process into brain tissue *in vivo* might be explained by the existence of specific carrier systems for amino acids in the blood-brain barrier (Oldendorf 1973) with a relatively slow transport rate for AIB (Oldendorf 1971).

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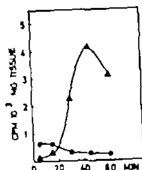


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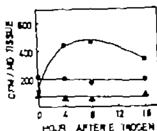


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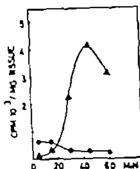


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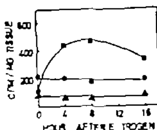


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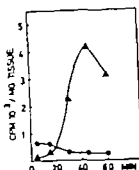


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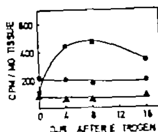


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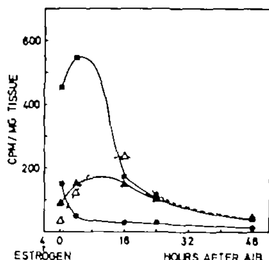


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References

CHRISTENSEN, H. N. *Biological Transport*, 2nd ed. 1975. W. A. Benjamin, Inc., Reading, Mass., U.S.A.

CHRISTENSEN, H. N. and M. E. HANDLOGTEN, Modes of Mediated Exodus of Amino Acids from the Ehrlich Ascites Tumor Cells. *J. biol. Chem.* 1968, *243*, 5428–5438.

GRIFFITHS, J. B. and S. J. PIRT. The uptake of amino acids by mouse cells (strain LS) during growth in batch culture and chemostat culture: the influence of cell growth rate. *Proc. roy. Soc. B.* 1967, *168*, 421–438.

HEDBERG, K. W. *The induction of implantation in the mouse after ovariectomy.* Stensels 1967, *10*, 581–601.

McCORQUODALE, D. J. and G. E. MUELLER, Effect of estradiol on the level of amino acid-activating enzymes in the rat uterus. *J. biol. Chem.* 1958, *232*, 31–42.

NOALL, M. W. and W. M. ALLEN, *Early stimulation by estradiol of amino acid penetration in rabbit uterus. J. biol. Chem.* 1961, *236*, 2987–2990.

NOALL, M. W., T. R. RIGGS, L. M. WALKER and H. N. CHRISTENSEN, Endocrine control of amino acid transfer. *Science* 1957, *126*, 1002–1005.

OLDENDORF, W. H. Brain uptake of radiolabeled amino acids, amines and hexoses after arterial injection. *Amer. J. Physiol.* 1971, *221*, 1629–1639.

OLDENDORF, W. H. Stereospecificity of blood-brain barrier permeability to amino acids. *Amer. J. Physiol.* 1973, *224*, 967–969.

OXENDER, D. L. and H. N. CHRISTENSEN, Distinct mediating systems for the transport of neutral amino acids by the Ehrlich cell. *J. biol. Chem.* 1963, *238*, 3686–3699.

PETERS, P. P. ROSELL and E.-E. BAULIEU, Steroid-induced early protein synthesis in rat uterus and prostate. *Europ. J. Biochem.* 1975, *60*, 137–145.

RIGGS, T. R. *Action of hormones on molecular processes.* Eds. Litwack, G. and D. Kritchevsky. Wiley & Sons Inc. New York 1964, 1–57.

RIGGS, T. R. and M. W. PAN, Transport of amino acids into the oestrogen primed uterus. Enhancement of the uptake by a preliminary incubation. *Biochem. J.* 1972, *128*, 19–27.

RIGGS, T. R., M. W. PAN and H. W. FENG, Transport of amino acids into the estrogen primed uterus. *Biochim. biophys. Acta (Amst.)* 1968, *150*, 9–103.

RIGGS, T. R., M. W. PAN and H. W. FENG, Transport of amino acids into the estrogen primed uterus. III. Effect of the level of extracellular sodium ion. *J. biol. Chem.* 1972, *247*, 7128–7134.

WALLIS, M. R., A. L. LAWRENCE and R. L. HAZELWOOD, Amino acid transport by the rat uterus during the estrous cycle and pregnancy. *Biol. of Reprod.* 1975, *12*, 383–388.

WEBBER, W. A. A comparison of the efflux rates of AIB from kidney cortex slices of mature and newborn rats. *Canad. J. Ph. and Pharmacol.* 1968, *46*, 765–769.

WEBBER, W. A. and J. A. CAMERON, A comparison of the amino acid concentrating ability of the kidney cortex of newborn and mature rats. *Canad. J. Physiol. Pharmacol.* 1968, *46*, 165–169.

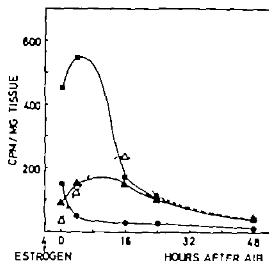


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References

Christensen, H. N., *Biological Transport*, 2nd ed. 1975. W. A. Benjamin, Inc. Reading, Mass., U.S.A.

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Finn, C. A. and K. W. ... The induction of implantation in the mouse after ovariectomy. *Steroids* 1967. *10*. 581–601.

McCorquodale, D. J. and G. E. Mueller, Effect of estradiol on the level of amino acid-activating enzymes in the rat uterus. *J. biol. Chem.* 1958. *232*. 31–42.

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Oxender, D. L. and H. N. Christensen, Distinct mediating systems for the transport of neutral amino acids by the Ehrlich cell. *J. biol. Chem.* 1963. *238*. 3686–3699.

Puvaneswari, P., P. Robel and E.-E. Baulieu, Steroid-induced early protein synthesis in rat uterus and prostate. *Europ. J. Biochem.* 1975. *60*. 137–145.

Riggs, T. R., *Action of hormones on molecular processes*. Eds. Litwack, G. and D. Kritchevsky. Wiley & Sons Inc., New York 1964. 1–57.

Riggs, T. R. and M. W. Pan, Transport of amino acids into the oestrogen primed uterus. Enhancement of the uptake by a preliminary incubation. *Biochem. J.* 1972. *128*. 19–27.

Riggs, T. R., M. W. Pan and H. W. Feng, Transport of amino acids into the estrogen primed uterus. *Biochim. biophys. Acta* (Amst.) 1968. *150*. 92–103.

Riggs, T. R., M. W. Pan and H. W. Feng, Transport of amino acids into the estrogen primed uterus. III. Effect of the level of extracellular sodium ion. *J. biol. Chem.* 1972. *247*. 7128–7134.

Walters, M. R., A. L. La... and R. L. Hazelwood, Amino acid transport by the rat uterus during the estrous cycle and pregnancy. *Biol. of Reprod.* 1975. *12*. 343–348.

Webber, W. A., A comparison of the efflux rates of AIB from kidney cortex slices of mature and newborn rats. *Canad. J. Physiol. Pharmacol.* 1968. *46*. 765–769.

Webber, W. A. and J. A. Cairns, A comparison of the amino acid concentrating ability of the kidney cortex of newborn and mature rats. *Canad. J. Physiol. Pharmacol.* 1968. *46*. ...

Acta physiol. scand. 1977 99 42–47

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Intra and Extracellular Activity of Ciliated Cells

By

D Huberman, C. H. Håkansson U Mercke and N G Toremalm

Received 13 May 1976

Abstract

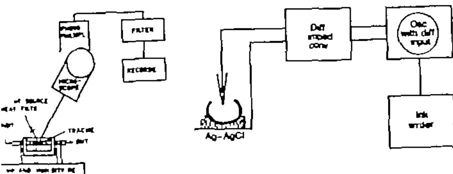
Huberman, D., C. H Håkansson, U Mercke and N G Toremalm *Intra- and extracell activity of ciliated cells* Acta physiol scand 1977 99 42–47

A method permitting simultaneous recording *in vitro* of the intracellular and extracellular (muc ciliary) activity of respiratory ciliated mucous membrane is presented. Using this method the influen of different temperature levels (20° 30° 40°C) upon the tracheal mucous membrane of 15 rabbits has be investigated. It is shown 1) that the extracellular (= mucociliary) activity increases with rising temperatu whereas the intracellular activity is unaffected and remains constant, indicating temperature influen upon the respiratory tract mucus rather than a change of the intracellular "pace-maker mechanis 2) that the restraining effect of the mucus decreases with rising temperatures but is still 10 % of the int cellular frequency at body temperature 3) that the presented method should be useful for future investiga tions concerning the effect of air pollution and pharmacological substances upon respiratory muco membranes

Key words: Trachea, mucous membranes, ciliated cells, mucociliary activity electrophysiological activi

The mechanical defence mechanism of the respiratory mucous membrane is constitut of two factors—the propulsive movements of the cilia and the more or less restricting effe of the covering layer of mucus. The term mucociliary activity has therefore been found mo adequate than the previously used designation, "the ciliary beat frequency (Mercke *et a* 1974 a). It has hitherto not been possible to separate the rhythm and frequency of intrins ciliary activity from that of the extrinsic mucociliary activity observed through a micr scope, but such a functional difference should be of great practical importance for exper mental and diagnostic purposes. However this is not possible with the use of extracellula recording methods only A combination of intra- and extracellular measurements is nece sary

The intracellular electrical activity in beating ciliated cells was first recorded in vertebrate by Håkansson and Toremalm in 1966. In recent experimental studies we found that the fre quency of intracellular action potentials was about the same at 20 30° and 40°C during *i vitro* conditions while the extracellular movements of the cilia decreased considerably wit decreasing temperatures (Toremalm *et al* 1975) This means that the frequency change a



g. 1 Block diagram of the experimental equipment for recording mucociliary wave frequency (left) and tracellular activity (right).

wer temperatures is due to physical properties of the secretion layer and not to the tracellular "pace-maker" mechanism. However this conclusion was drawn from recordings f the intracellular and extracellular activities in separate specimens. Simultaneous recordings from the same area would be even more valuable for theoretical and practical purposes. The aim of the present investigation has therefore been, 1) to device method for the simultaneous recording of the intracellular electrical activity and the extracellular mucociliary ave frequency in the same area on tracheal specimens and 2) to compare the frequencies of the electrical discharge and the mucociliary movements at different temperature levels within the range of 20–40 C

Methods and material

Rabbits weighing 1.5– 0 kg have been used as experimental animals. T void undesired pharmacological side effects they have been sacrificed by blow on their heads. After careful dissection of the trachea 5 4.0 cm specimen has been removed and put into an experimental chamber in hich temperature and relative humidity can be regulated and recorded.

Recording of the mucociliary act ity

The recording technique has already been extensively described (Mercke et al. 1974 a). Shortly cold light beam directed against the tracheal ciliated mucous membrane through slit in the upwards directed part membrane. Variations of the light intensity of the mucosal reflex are picked up by microscope Fig. 1 A photomultiplier EMI 9524 B) attached to one of the oculars converts the light intensity variations to electrical signals hich after amplification and filtering (Krone Hite type 3550) are recorded by an ink iter (Elema Mingograph 34)

Recording f the intracellular lectrical activity

The technique of recording is modification of the method originally described by Håkansson and Toremalm in 1966 and has in detail been described by Toremalm et al (1975) In the actual investigation further improvement has been introduced by exchanging the formerly used transistorized impedance converter to low frequency impedance converter ith resistance of 1 000 MΩ and an amplification of 1 1 Fig. 1

Experimental proc dure

After the tracheal specimen has been placed into the experimental chamber the capillary microelectrode is ery slowly pushed downwards through the mucus into the cell layer in the center of the light reflex. The oscillos pe is in the DC position. When potential change of 15 to 40 mV indicates that ciliated

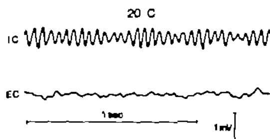


Fig. [illegible] Recordings from one tracheal specim showing the intracellular activity (IC) and t mucociliary wave pattern (EC) at 20°C.

cell membrane has been penetrated, the oscilloscope is changed to the AC position and the abov describ intracellular recording is commenced. The microscope is then adjusted so that variations of the light refl tions exactly corresponding to the area of intracellular recording may be picked up and recorded.

The simultaneous recording of intracellular electrical and extracellular mechanical acti ities from t very small mucosal area has been performed at 3 different temperature levels (20° 30° and 40 C) and an environmental air humidity above 90%. The trachea from 5 rabbits h ve been investigated at ea temperature level.

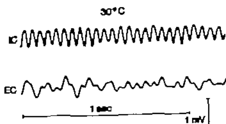
Results

At 20°C (Fig. 2) the intracellular potential recordings show a fairly even rhythm and frequency of 20 cycles/s whereas the extracellular recordings are irregular with a low fre quency of about 12 waves/s (Table I)

At 30°C (Fig. 3) the intracellular frequency does not change in contrast to the extracellul activity which has increased. The difference in the intra and extracellular activities

TABLE I

Temp. (°C)	Intracell lar activ ty (oscillations/s)	Extracellular (mucociliary) acti ty (waves/s)
20	20 5	9 6
	21 5	13.1
	21.0	17 1
	21 7	10.4
	21.3	12.7
	M 21.2	M 12.6
30	21.3	16.7
	21.5	17 4
	23 1	18.0
	21 4	17 6
	20.8	16.3
	M 21 6	M 17 2
40	22.1	18.8
	22.0	19 5
	21 5	17 7
	21.2	18.8
	21.5	17 4
	M [illegible]	M [illegible]



3. Recordings from one tracheal specimen showing the intracellular activity (IC) and the mucociliary wave pattern (EC) at 30°C.

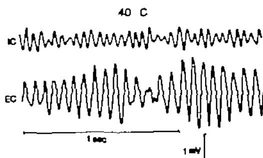


Fig. 4. Recordings from one tracheal specimen showing the intracellular activity (IC) and mucociliary wave pattern (EC) at 40°C.

markedly reduced (20 cycles and 17 waves/s respectively Table I). At 40°C (Fig. 4) this difference is even more reduced, now being 20 cycles and 18 waves/s respectively (Table I). The frequency/temperature relationship concerning the intracellular as well as the extracellular recordings is illustrated in Fig. 5 The plotted values are the mean frequencies of 5 rabbit specimens at each temperature level (Table I). *From this figure it is clearly evident* that the intracellular activity is mainly unaffected by temperature changes in the interval 20 C to 40 C However the extracellular or mucociliary activity increases almost linearly with increasing temperature in the same interval.

Discussion

A comparative study of the intracellular electrical activity which initiates the movements of the cilia covering the surface of the respiratory tract, and the extracellular manifestations

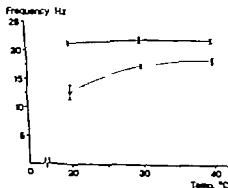


Fig. 5 The frequency temperature relationship regarding intracellular (broken line) and extracellular (dotted line) activities simultaneously measured. Each plotted value the average of 5 measurements.

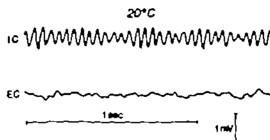


Fig. 2. Recordings from one tracheal spe showing the intracellular acti ity (IC) a mucociliary wave pattern (EC) t 20°C.

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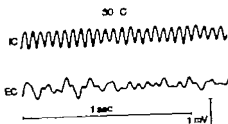
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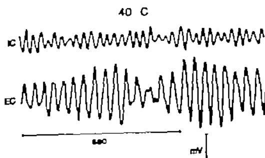
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	21 4	17 6
	20.8	16.3
	M 21 6	M 17 2
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	21.5	17 7
	21.2	18.8
	21.5	17 4
	M 21 5	M 18 4



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Discussion

A comparative study of the intracellular electrical activity which initiates the movements of the cilia covering the surface of the respiratory tract, and the extracellular manifestations

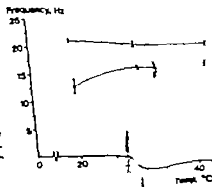


Fig. 5. The frequency temperature relationship regarding intracellular (broken line) and extracellular (dotted line) activities simultaneously measured. Each plotted value is the average of 5 measurements.

of this activity is presented in this investigation The experimental model is a comb of two previously described methods (Fig. 1) dealing with one of these activities at (Mercke *et al* 1974 a Toremalm *et al* 1975) It has been possible to make simult recordings of the intracellular electrical activity in one ciliated cell and the extrac mechanical activity from a small area covering about 10 ciliated cells during stand conditions regarding temperature and humidity of the surrounding air

This investigation has been limited to a comparison of the two mentioned activ three different temperature levels. Typical recordings at 20° 30° and 40 C are demon in Fig. 2, 3 and 4 respectively The entire results regarding all tracheal specimens are in Table I and are also graphically illustrated in Fig. 5

The relative constancy of the intracellular oscillations from 20 to 40°C is a physiolo interesting phenomenon. In fact this may have two explanations 1 The rhythm "pace-maker" is relatively insensible to temperature changes. 2. The frequency n constant but the rising time of the potential fluctuation can be shorter and the ris rapid at higher temperatures. This may result in an increasing amplitude of the intrac recording. Such an explanation cannot be deduced from Fig. 2, 3 and 4 since the rec at 20, 30 and 40 C are obtained from different tracheal specimens. To be able to the hypothesis furthe. expts. have to be done where one tracheal specimen is inves at the different temperatures.

The extracellular mucociliary activity on the other hand is strongly influenced by te ture changes. This has been described more in detail in two previous papers (Merck 1974 b Mercke 1974). From the present simultaneous recordings frequency diffe between the intra and extracellular activities increase by decreasing temperature below 40 C (Fig. 5). Ordinary body temperature therefore seems to be optimal for th to work in. In other words the mechanical restriction of the secretion layer is mini this temperature level. However even at optimal temperature conditions there is a diff of about 2 cycles/s between the internal and external activities. It means that the sec layer normally has a decelerating effect upon the movements of cilia. The consequ of the present results are (1) that the method makes it possible to separate the intrac ciliary activity (the "pace-maker" effect) from the retarding effect of the mucus layer surr ing the cilia, and (2) that this inhibitory factor reduces the frequency by about 10 above body temperature.

This knowledge seems to be valuable for future experimental investigations in whi model may be used *e.g.* for air pollution tests and control of pharmacological subs for local treatment of the respiratory tract From the results a hypothetical conclusio be drawn implying that it must be easier to stimulate the mucociliary defence mech by changing the physical properties of the mucus than to try to accelerate the intrac activity of the ciliated cells. However this hypothesis need to be proven by a series of fu expts. which are now in progress.

This investigation has been supported by grants from the Swedish Medical Research Council, nr B75-17X 3897-03A.

References

…ANSSON, C. H. and N. G. TOREMALM, Studies on the physiology of the trachea. III. Electrical activity of the ciliary cell layer. *Ann. Otol.* (St. Louis) 1966, *75* 1007–1018.

…ECKE, U. The influence of temperature on mucociliary activity. Temperature range 40 C–50°C. *Acta oto-laryng.* (Stockh.) 1974, *78*, 253–258.

…ECKE, U., C. H. HÅKANSSON and N. G. TOREMALM, A method for standardized studies of mucociliary activity. *Acta oto-laryng.* (Stockh.) 1974 a, *78*, 118–123.

…ECKE, U., C. H. HÅKANSSON and N. G. TOREMALM, The influence of temperature on mucociliary activity. Temperature range 20 C–40°C. *Acta oto-laryng.* (Stockh.) 1974 b, *78*, 444–450.

…MALM, N. G., C. H. HÅKANSSON, U. MERCKE, B. DAHLERUS and D. HUBERMAN, Mucociliary …ation. A comparative analysis of intracellular and extracellular activity. *Acta oto-laryng.* (Stockh.) …975, *79*, 436–441.

Acta physiol. scand. 1977 99 48–52
From the Institute of Physiology University of Lund, Sweden

The Effect of Teeth Amputations on the Choline Acetyltransferase Activity of Rat Submaxillary Glands

By

JÖRGEN EKSTRÖM

Received 21 May 1976

Abstract

EKSTRÖM, J *The effect of teeth amputations on the choline acetyltransferase activity of rat submaxillary glands* Acta physiol scand. 1977 99 48–52.

The choline acetyltransferase activity in parasympathetically decentralized glands was unaffected by repeated teeth amputations over a period of 2 weeks, while after the same period of time the treatment caused the enzyme activity to increase in innervated glands. It appears that the enzyme in the postganglionic nerve is for its activity dependent on an intact connection with the central nervous system. The increase in enzyme activity is attributed to an enhanced reflex stimulation of the glands from pulpal receptors.

Key words Choline acetyltransferase activity salivary glands, impulse flow

When the parasympathetic postganglionic neurones of the salivary glands loose their connection with the central nervous system by a section of the preganglionic nerve supply their capacity to synthesize acetylcholine has been found to diminish, as judged from the fall in the activity of choline acetyltransferase (Nordenfelt 1964 Ekström and Holmberg 1972). Based on this observation Nordenfelt (1964 1965) suggested that the activity of the acetylcholine forming enzyme might be influenced by the intensity of the impulse traffic in the neurones as to the adrenergic neurones a similar relationship between the activity of catecholamine synthesizing enzymes and impulse traffic has since been considered (Zieher and Pellegrino de Iraldi 1966, Molinoff and Axelrod 1971 Thoenen 1974) The idea put forward by Nordenfelt is favoured by the outcome of various types of experiments on salivary glands, where the degree to which the glands were activated from the central nervous system was either decreased or increased over a long time period (see Ekström 1975) in parotid glands of rats the choline acetyltransferase activity declined when the animals were kept on a liquid diet, while it increased when the diet was dry and bulky or when the mouth and the throat of the animals were made dry by atropinization or salivary duct ligations. The increase in the choline acetyltransferase activity in the submaxillary glands of the rat after repeated teeth amputations reported by Ohlin and Perec (1967) seems also to be a finding which would fit into the pattern outlined above the enzyme activity is increased as a consequence

…e enhanced stimulation of the glands reflexly elicited by the irritation of pulpal receptors. …ever these authors reported that in the parasympathetically decentralized glands the …ne acetyltransferase activity had increased after the amputations. This might suggest … some other factor than the impulse traffic is responsible for the increased enzyme …ity seen after teeth amputations.

…n the present study the effect of teeth amputations on the choline acetyltransferase …vity was compared in decentralized and innervated submaxillary glands.

Methods

…ale rats of Sprague-Dawley strain bred at this Institute were used. They …ere at the start of the expts. … months old. The diet used …as of … gruel-like consistency prepared daily by mixing … powder made of …tandard pelleted diet …ith water, so as to make it easier for the rats …ith amputated teeth to eat; the …s serving as controls …ere also given this diet. *The diet and water …ere given ad libitum.* Both at the start …d at the end of the experimental period the body …eights of the rats were determined.

…e rats lightly anaesthetized …ith ether both the lower and upper incisors were cut to the gingival margin …h … bone cutting forceps every other day over … period of either 14 or 21 days. The rats serving as controls …re also exposed to ether for … few minutes at the same occasion.

In one type of expt. the chorda-lingual nerv… was bilaterally cut under … dissecting microscope in 18 …s, and … few mm of the nerv… …as removed. The wound was then sutured. I… 9 of these rats teeth amputa…ns started 7 day… later and continued over … same period of 14 days, the other 9 rats …ere litter mates to … amputated ones and served as controls. In another type of expt. rats …ith an intact innervation of their …ivary glands were exposed to the amputations. I… … first series, the incisors of 9 rats were amputated …er … period of 14 days, in … second series 7 rats were amputated over … period of 21 days, 9 and 7 …ated litter mates to those …hich were amputated served as controls.

At the end of the experimental period the rats were killed by inhalation of ether and the submaxillary …nds were removed (the sublingual glands being separated from the submaxillaries), …ashed in saline, …ared, pressed gently between gauze pads and weighed. In the rats studied over the period of 3 weeks …e sublingual glands were also removed and treated in the same …ay as the submaxillaries. Both wet …d dry weights were determined, the latter … expressed as acetone dried powder.

The glands were then prepared for the analysis of their activity of choline acetyltransferase using the …ethod devised by Hebb (see Nordenfelt 1963, 1965). Acetone dried powder …as made of the 2 pooled …ubmaxillary glands of one rat, as to the sublingual glands each pool consisted *of the glands of 7 rats.* The powder of the glands …as made up in cysteine-saline in … concentration of 50 mg/ml. Of the tissue …xtract 0.2 ml …as incubated at 38 C for 60 min. Experimental preparation and control preparation …ere …ncubated simultaneously and the assay for acetylcholine was made on the same eserinized frog rectus. The choline acetyltransferase activity is expressed in µg acetylcholine chloride formed per h per pooled glands (total activity) and in µg acetylcholine chloride formed *per h per g acetone powder (concentration).*

Student's t-test …as used, paired comparisons …ere made between the experimental rat and its control litter mate. P …alues of less than 0.05 were considered significant.

Results

Body …eights. At the start of the expts. no differences existed in body weights between the …ats to be used as controls and those to be teeth amputated. The body weights of the teeth amputated rats with decentralized glands decreased (p … 0.001) during the 14 days from …mean … S.E.) 294 … 8 to …59 … 8 g (n … 9), i.e. by 12 …. During the same time period the control litter mates increased (p … 0.001) in weights by 7 …, from 293 … 9 to 314 ± 9 g (n … 9). The body weights of the teeth amputated rats with an intact innervation decreased from 301 … 7 to …67 … 8 (9) g, … e. by 11 … in the expt. of the duration of 14 days and in the expt.